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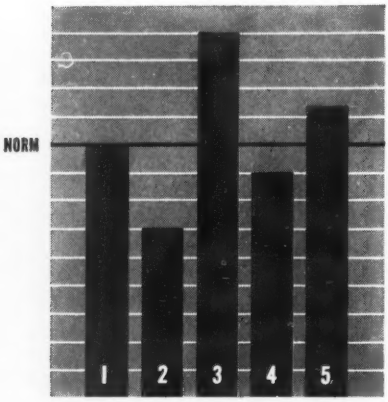

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THE INTERSTITIAL TISSUE OF A HUMAN HERMAPHRODITE*

ERNEST VAN CAMPENHOUT, M.D. AND EMIL WITSCHI, PH.D.
From the Anatomy Department, University of Louvain, Louvain, Belgium, and the Zoology Department, University of Iowa, Iowa City, Iowa

THE site of production of the sex hormones in human males is still a disputed question. In a recent contribution by Witschi and Mengert (1) evidence is presented supporting the contention that the sustentacular cells of the seminal tubules (so-called cells of Sertoli) are the producers of the female sex hormones. In a patient of feminine appearance, without ovaries, but with large testes, the urine contained a considerable amount of estrogens. After removal of the sex glands, the estrogenic substances disappeared. A histologic study proved the complete absence of ovarian cortex and an abortive condition of spermatogenesis. The interstitial cells (of Leydig) were small and did not give the impression of much activity. On the other hand, the sustentacular cells were highly developed, and gave the appearance of intense secretory activity. The morphologic homology of the ovarian granulosa and the sustentacular cells of the testis was stressed, and the origin of the female sex hormones of this patient was attributed to the sustentacular cells.

During the last few years, one of us (Van Campenhout (2, 3, 4, 5)) became interested in the neural relationships of the interstitial tissue in human sex glands, and presented new evidence in favor of the interstitial nature of sympathicotropic cells described by Berger (6). As in human testes and ovaries, intimate connections between interstitial cells of Leydig and nerve

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fibers were also found in the testes of some primates. According to these observations, neuro-epithelial complexes are present in the gonads of both sexes. Since the interstitial cells of the testes and the cells of the theca interna of the ovary are homologous, a similar function might reasonably be expected to exist in these gonads. It was suggested that the nervous

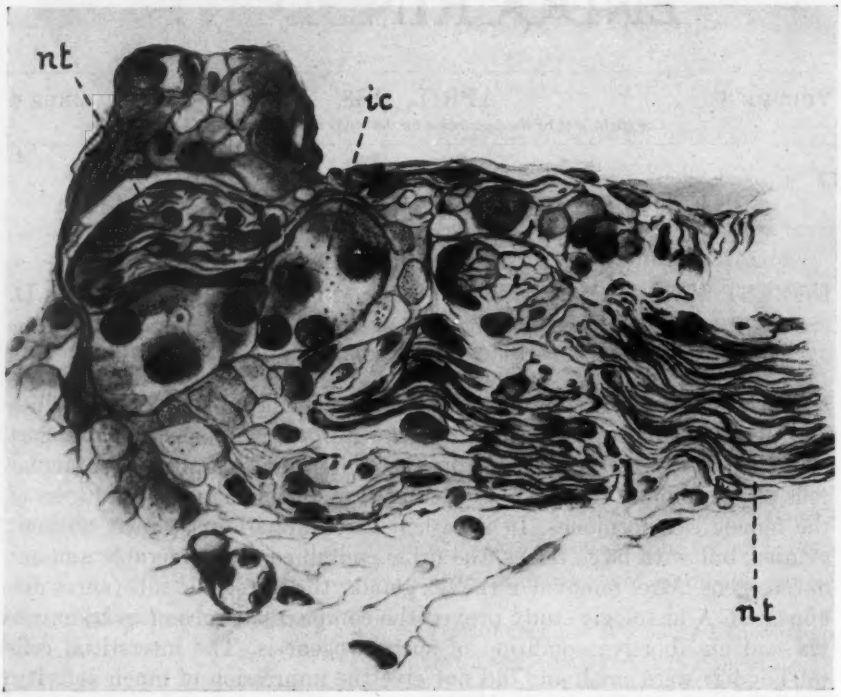

FIG. 1. Part of albuginea from section through hermaphrodite gonad. Nerve trunk (n.t.) in close association with a group of interstitial cells (i.c.). Some nerve fibers pass in between these cells. Stained by the trichromic method of Masson.

stimuli might modify the normal secretion of the homologous elements of the gonads so as to give rise to different hormones.

It was, therefore, decided to make a renewed study of the sections of the sex glands removed from the patient and to test, in this possibly crucial case, the validity of the last mentioned hypothesis. Absence of neuro-epithelial complexes would constitute a strong argument against it.

Some of the previously prepared sections were bleached with acetic alcohol or sulphuric bichromate and restained by Masson's trichromic process. This method gives a clearcut differentiation of nerve and connective tissue fibers.

The observations may be described briefly. The interstitial elements between the seminal tubules are essentially identical with those of the albuginea. The cytoplasm does not show any marked difference between endoplasmic and ectoplasmic zones; it contains few vacuoles. For these reasons one may consider them as being in a relatively inactive state. Of course, it is not certain that endo-ectoplasmic differentiation or vacuolization are indispensable aspects of endocrine activity, and the possibility of some degree of hormone production by these interstitial elements is not

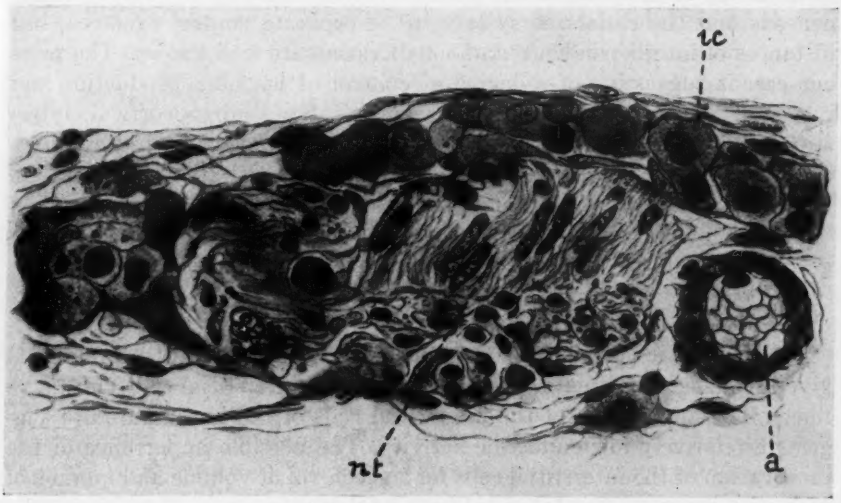

FIG. 2. Detail from another section; same material and technique. Numerous interstitial elements (i.c.) are enveloping a nerve trunk (n.t.) in which a few Schwann cells are seen, *a* artery. Both figures about ×750.

denied. In fact, it is perfectly obvious that before the operation small amounts of androgenic hormones were available, which maintained epididymides of normal male size and of good histologic development.

In the albuginea one finds numerous instances of intermingling or interpenetration of interstitial cells and nerve fibers. Typical areas of restained sections are reproduced in Figures 1 and 2. Both show the intimate interrelationship of interstitial cells and amyelinic nerve fibers, the latter twisting between and around the epitheloid elements. The images are very similar to those presented by hundreds of neuro-epithelial complexes which were observed in normal and cryptorchid males, so that there can be no doubt about the neural nature of these fibers. There is but one difference, and that concerns the aspect of the cytoplasm of the cells of Leydig. In normal and cryptorchid males, many sympathicotropic cells show either a

differentiation of endo- and ectoplasm, a vacuolized aspect of the cytoplasm, or more or less numerous inclusions of crystalloids (of Reinke). But in the present case, the cytoplasm stains uniformly and appears of even density, without special inclusions in the majority of the cells. As stated above these histologic features apparently are related to a low secretory activity of the interstitial cells, and possibly to the low androgen level of this patient.

The reported observations raise the question of the functional importance of the innervation of the interstitial cells. Usually one thinks of the nervous and the endocrine systems as of separate control agencies; but instances of interdependence and collaboration are well known. The present case is suggestive of a degree of control of hormone production and release by the autonomous nervous system. Since adrenocortical, interstitial, and thecal cells are closely related, embryologically and cytologically, one may consider qualitative as well as quantitative modification. Okkels and Sand (7) even hint at the possibility of a separate secretion of "still unknown kind" by the Berger cells. The actual value of these suggestions remains to be tested by experimental and physiologic methods.

CONCLUSIONS

The histologic study of the sex glands of a human hermaphrodite (1) gives evidence of intimate connections between interstitial cells and nerve fibers. The appearance of the interstitial cells of the hermaphrodite suggests a relatively low endocrine activity. The possible importance of the innervation of the interstitial cells for the control of volume and quality of endocrine production is taken into consideration.

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ADRENAL AND TESTICULAR DEFICIENCY

A COMPARISON BASED ON SIMILARITIES IN ANDROGEN DEFICIENCY, ANDROGEN AND 17-KETOSTEROID EXCRETION, AND ON DIFFERENCES IN THEIR EFFECTS UPON PITUITARY ACTIVITY

E. PERRY McCULLAGH, M.D., ROBERT W. SCHNEIDER, M.D., WENDELL BOWMAN, PH.D.* AND M. B. SMITH, M.D.

Cleveland Clinic, Cleveland, Ohio

ACCORDING to the theory that there is a second testicular hormone (inhibin), deficiency of this factor, as in the human castrate or in severe testicular failure, is chiefly responsible for the ensuing pituitary hyperactivity. It is generally conceded that androgens arise in the cortex of the adrenal gland as well as in the Leydig cells of the testes. The androgens arising in the testes, together with the androgens and other related substances arising in the cortex of the adrenal, are largely represented in the urine as a group of substances known as 17-ketosteroids. Measurement of these is frequently used to estimate certain hormonal activities of the testes and the adrenal glands. Androgenic activity *per se* is measurable biologically.

In women with ovarian deficiency the cause of pituitary hyperactivity apparently is the loss of estradiol. The high levels of urinary follicle-stimulating hormone (F.S.H.) occurring after castration or the menopause can be reduced by small amounts of estradiol or other estrogens. In Addison's disease in women the loss of androgens from the circulation is apparently the cause of the marked loss of body hair and is indicated also by the extremely low levels of 17-ketosteroids in the urine. It has not been shown that this loss of androgens is followed by increased activity of the anterior pituitary gland, as judged by an increase of urinary F.S.H. The fact that there tends to be a disappearance of basophil (sex hormone-producing (1)) cells from the anterior lobe under such circumstances would lead to the suspicion that the opposite might be the case.

The fact that pituitary hyperactivity occurs in the male as a result of castration or of severe primary testicular failure seems to be acceptable. The occurrence of such high levels of urinary F.S.H., in castrate or eunuchoid men, as shown later, is evidence of this point. Some workers have

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* Formerly in the Research Division, Cleveland Clinic, Cleveland, Ohio.

ascribed pituitary hyperfunction resulting from testicular deficiency solely to the deficiency of androgens which exists. Evidence which has accumulated in the past, as well as data presented here, strengthen the opinion that this is not a fact, and that a deficiency of some other hormonal activity is partly and probably chiefly responsible.

A few of the arguments regarding a testicular hormone arising in the germinal epithelium may be mentioned. The idea of an independent endocrine function of the germinal epithelium of the testis was first advanced by Mottram and Cramer (2) in 1923. They showed that application of radium to the testes of the rat produced little effect upon the interstitial cells but rather marked atrophy of the gametogenic elements. Under these circumstances there occurred histologic changes in the anterior lobe of the hypophysis similar to those found after castration. Because the secondary sex characteristics are not obviously affected by destruction of the germinal epithelium, the production of two hormones was suggested by Martins and Rocha (3).

McCullagh (4) and McCullagh and Walsh (5) found that aqueous extracts of the testis caused disappearance of castration changes in the hypophysis, although they brought about no apparent androgen-evoked change in the accessory sex glands. McCullagh and Walsh (5) showed that this effect remained after the lipoid-soluble substances had been removed from such testicular extracts. Roy McCullagh (4) named this water-soluble, pituitary-inhibiting testicular substance "inhibin."

Nelson's (6) studies of cryptorchid animals led him to the belief that castration changes in the pituitary in the presence of damage to the germinal epithelium, are the result of a great sensitivity on the part of the hypophysis and that castration effects of a mild degree did occur in the accessory organs. He felt that such changes need not necessarily be explained on the basis of a second hormone.

Since many workers have shown that sufficiently large doses of androsterone and testosterone, as well as estrogens, will prevent or reverse castration changes, Moore (7) and others have considered the existence of a second testicular hormone improbable.

Törnblom (8) in Uppsala has made extended and well controlled experiments along these lines. His work supports the idea that a second testicular hormone exists. Among other arguments he shows that "giving testosterone to young castrate male rats in such quantity that the weight of the prostate and seminal vesicles somewhat exceeds the weight of these organs in normal animals will not prevent the increase in weight set up in the hypophysis after castration." Such results can readily be duplicated, as shown by the following data from our own laboratory.

	No.	Body Weight	Pituitary	Prostate	Seminal Vesicles
		Gm.	mg.	mg.	mg.
Noncastrate controls	1	255 approx.	6.4	257.0	461.2
	2	258 approx.	6.4	289.2	482.8
	3	260 approx.	5.8	164.8	242.4
	4	262	6.8	282.2	338.6
	5	255	6.2	252.0	392.0
	6	257	7.8	311.6	410.0
Castrated controls	1	290	12.0	atrophied	78.8
	2	280	11.6	atrophied	100.0
	3	287	10.8	atrophied	90.0
	4	255	12.0	none	100.2
	5	278	10.2	atrophied	134.6
	6	272	11.0	atrophied	102.0
Castrate-testosterone injected, 100 micrograms daily	1	318	11.2	433.8	652.4
	2	277	11.4	351.9	509.2
	3	292	8.2	496.6	511.6
	4	305	9.2	321.9	515.6
	5	294	10.4	361.2	486.6
	6	282	10.2	310.0	490.0
Castrate-estrololactone injected, 100 micrograms daily	1	300	14.8	none	131.9
	2	273	13.2	19.0	96.6
	3	276	12.8	atrophied	107.4
	4	300	10.0	29.0	94.8
	5	256	10.0	atrophied	108.0
	6	295	12.0	atrophied	124.0

Törnblom extracted a fraction from bull testes which inhibits the weight increase in the hypophysis of castrated male rats. He calls attention to the fact that this fraction has biologic activities which tally closely with estradiol.

In man two factors may lead to the production of high titres of urinary gonadotropins: 1) castration or severe total testicular damage; 2) oligospermia, in some instances of which there is no clinical evidence of androgen deficiency and in which normal 17-ketosteroid excretion exists. In the latter instance the high gonadotropin excretion cannot be explained on a basis of Leydig cell failure alone and is probably the result of inhibin deficiency. Examples of this are seen in men with cryptorchidism who are large, strong, and normally aggressive, with a normal voice, beard, body

hair, external genitalia and prostate. In other words, these men have not the slightest suggestion of androgen deficiency. Assays for urinary 17-ketosteroids are normal. They have inactive tubular tissue and high titres of urinary F.S.H. There are also such patients as those described by Klinefelter et al. (9) who have oligos- -mia and gynecomastia, normal urinary 17-ketosteroids, and high urinary F.S.H. These men probably are also examples of deficiency of a second testicular hormone resulting in pituitary hyperfunction, though it is not to be overlooked that these patients merge in clinical appearance with those showing mild eunuchoidism and their appearance does not differ materially from the hypogonadal group with gynecomastia described by Nelson and Heller (10). Data of our own soon to be published indicate that in the presence of testicular deficiency, large doses of androgens fail to reduce the high levels of urinary gonadotropins. It appears also that a defect in gametogenic elements is more intimately connected with such a change than a defect in Sertoli's cells, since similar high titres of gonadotropins may be found in patients whose tubules contain only Sertoli's cells.

It occurred to us that if it were true that diminution of androgens in the body were alone responsible for the pituitary hyperactivity, and if it were also true that androgens in both men and women arise in the adrenal gland, then it should follow that high titres of urinary gonadotropins should be present in patients with destroyed adrenal glands, as in Addison's disease. This should be especially true in women since in them, the only source of androgens is impaired or lost.

In using this type of comparison of testicular and adrenal disease two questions might be raised. First, in testicular failure are the clinical evidences of androgen deficiency, the lowered excretion of androgens and 17-ketosteroids, due to deficiency in testicular hormone directly? From the large amount of experimental and clinical evidence available it appears that in primary testicular deficiency this is true. Certainly there is no evidence to suggest that pituitary or adrenal failure exists to explain such changes. In male rats castration leads to pronounced hypertrophy of both the adrenal cortex and the pituitary (11, 12). Clinically the higher urinary gonadotropins strongly suggest a high degree of pituitary function. In those patients in whom pituitary failure is associated with a pituitary tumor or other local pituitary lesion there are, besides the hypogonadism, other evidences of disease, including signs of failure of thyroid and adrenal functions. This makes it possible to separate these patients from those with primary testicular failure. The second question is: Does the adrenal failure in Addison's disease, with the loss of adrenal hormones, result directly in the diminished androgen effects and excretion and the diminished 17-ketosteroid excretion, or are these the result of associated phenomena? It

might be asked whether such changes are due to pituitary deficiency, and associated testicular failure in men, or to the general debility present.

Pituitary deficiency might be considered as a possible cause of Addison's disease in instances in which adrenal atrophy and not infection is present. If this were true, part of the changes under consideration might be due to testicular deficiency existing as part of Addison's disease. This explanation is not valid because: (a) patients with Simmonds' disease or hypopituitarism with pituitary tumor, though they have adrenal hypofunction, are clearly separable clinically from those with Addison's disease; (b) urinary 17-ketosteroids in patients with hypopituitarism are at a range approaching zero and are much lower than in men with Addison's disease (13); (c) relatively normal sperm counts which exist in Addison's disease indicate active pituitary function; (d) the high gonadotropin titre present in postmenopausal women with Addison's disease is good evidence of strong pituitary function.

The facts strongly favor the idea that the prostatic changes and the lowered androgen and steroid excretion seen in Addison's disease are directly due to a loss of adrenal hormones rather than to testicular deficiency. The prostatic atrophy frequently present in Addison's disease is evidently due to androgen deficiency. Such androgen deficiency is probably not due to testicular hypofunction for the following reasons: (a) testicular atrophy is not usually present in Addison's disease; (b) there are no symptoms of testicular deficiency; (c) sperm counts, though low in some patients, may be entirely normal in others; (d) we have been unable to demonstrate high urinary F.S.H. in Addison's disease except where gonadal failure exists, as in postmenopausal women, or in cases of complete aspermia (case 7) or severe oligospermia.

Low levels of 17-ketosteroid excretion have been observed in Addison's disease when the general health was good due to long continued treatment. This seems to indicate that the low excretion of these materials cannot be explained on the basis of debility.

Androgenic substances and 17-ketosteroids have been shown to be present in the adrenal cortex (14, 15, 16, 17, 18). It is well known that a definite increase in masculinizing materials may arise here in pubertas praecox in boys and in certain adrenal cortical tumors. Loss of these substances from the adrenal itself in Addison's disease seems clearly indicated by the extremely low levels of urinary 17-ketosteroids seen in women with this condition.

METHODS

17-Ketosteroid determinations. Twenty-four hour specimens of urine were used, with the exception of some pooled forty-eight hour specimens. The urine was acidified by the addition of 5 cc. of concentrated sulfuric acid per

100 cc. of urine and immediately hydrolyzed by boiling for ten minutes. The hydrolyzed urine was then extracted in three half-hour periods with redistilled butyl ether, 200 cc. per liter of urine being used for each extraction. The combined butyl ether extracts were washed several times with 1.5 N sodium hydroxide solution, followed by several washings with tap water until the ether layer was clear. In cases in which capon assay was done, half the extract was reserved for that purpose.

Capon assays were done on crude neutral fractions according to methods routinely used in this laboratory (19, 20), five test birds and five controls being used for each test. The butyl ether was removed by vacuum distillation and the residue dissolved in ethyl ether and finally taken up in sesame oil for injection.

The other half was divided into two fractions for 17-ketosteroid determination, earlier tests being made by the Girard (21) method and later ones by adsorption on magnesium oxide (22). Colorimetric determinations were made by the Holtorff-Koch modification (23) of the Zimmermann reaction (24, 25).

The gonadotropic hormone assays were done by one of two methods. In the rat uterine-weight method used in the earlier work (26) the entire twenty-four hour specimen is precipitated with four volumes of 95 per cent alcohol and fractionated according to the method of McCullagh and Bowman (27). The results are expressed in terms of actual rat uterine weight in milligrams.

The mouse uterine-weight method for gonadotropic hormone assays was that of Klinefelter, Albright, and Griswold (28). Immature female mice are used and the gonadotropin to be tested is precipitated from the urine by alcohol. One unit is considered the amount of the hormone which, when injected in five doses over a three-day period in a dilution of 0.5 cc. per dose, causes a minimal uterine weight increase. The uterus is considered enlarged if it weighs more than 7 mg.

RESULTS

Urinary 17-ketosteroids. Urinary 17-ketosteroids in a group of 40 normal men ranged from 4.9 to 18.4 mg. (mostly from 6 to 14 mg.) per twenty-four hours, and in 18 normal women, from 1.5 to 9.7 mg. per twenty-four hours (Table 1).

The range of results of individual tests set forth in Table 2 shows that urinary 17-ketosteroid levels in 13 men with Addison's disease, 5 castrate men, and 6 eunuchoid men, were lower than the normal range established in this laboratory, except in one assay in a man with Addison's disease (*case 1*). It should be noted that the results of the Girard method were usually somewhat higher than those obtained by the magnesium oxide

adsorption method. Results of individual tests in 5 women with Addison's disease (Table 10) were lower than those in men with Addison's disease; this fact is consistent with the generally held idea that since the only source of 17-ketosteroids in women is the adrenal cortex, women with Addison's disease excrete practically no 17-ketosteroids.

Urinary androgens. Levels of androgen excretion in normal individuals are given in Table 3. The results of 41 assays in 20 men are shown. Androgen assays (Table 4) in castrate, eunuchoid, and Addison's disease groups were

TABLE 1. URINARY 17-KETOSTEROIDS* IN NORMAL MEN AND WOMEN 20 TO 40 YEARS OF AGE

Men 40 Patients		Women 18 Patients	
MgO Adsorption		MgO Adsorption	
mg./24 hr.	No. of Cases	mg./24 hr.	No. of Cases
6.0†	4	1.0†	3
7.0	7	2.0	0
8.0	6	3.0	7
9.0	6	4.0	1
10.0	4	5.0	1
11.0	5	6.0	3
12.0	1	7.0	0
13.0	2	8.0	2
14.0	2	9.0	1
15.0	0		
16.0	1		
17.0	1		
18.0	1		

* All 17-ketosteroid assays in this and the following tables represent pure ketonic fractions.

† Each figure represents assays at that level and to 0.9 mg. above it.

abnormally low, with one exception (*case 29*); the levels in some of the tests in this patient fell within the low limits of normal. It is interesting that this patient was probably the most severely eunuchoid in the group. The majority of the assays in patients with Addison's disease were about half of the low limits of normal. In general, these figures are similar to those reported by Callow (29) who found levels of 7, 18, 23, 26, 9, 39, 13, 1, 5 and 20 international units in assays done on 7 patients with Addison's disease. One assay in a patient with Addison's disease after a two-year period of treatment (*case 5*) fell well within normal range, the figures com-

TABLE 2. URINARY 17-KETOSTEROIDS IN MEN WITH TESTICULAR FAILURE AND MEN WITH ADDISON'S DISEASE

Castrate		Eunuchoid		Addison's Disease		
					Method	
Case	mg./24 hr.	Case	mg./24 hr.	Case	MgO	Girard
					mg./24 hr.	mg./24 hr.
21	6.0	27	8.0	1	3.4	9.0
22	2.0		5.0	2		4.0
	2.0	28	6.0	4		4.0
	1.1*	29	5.0	5		4.0TNF†
	1.0*	30	6.0	6	2.8	5.0
	0.8*	31	8.0TNF†	7	2.5	6.3
	1.3*	32	2.0	8	1.7	
23	5.0			9	2.0	1.0
	4.0*			10		3.0
25	4.0			11		2.0
	3.0*			12		5.0
26	1.0			13	4.0	

* Fractionated by MgO adsorption; others by Girard separation.
† TNF = Total Neutral Fraction.

paring closely with those reported by Koch (30). In neither the castrate nor the Addison's disease group were the 17-ketosteroid levels and andro-

TABLE 3. ANDROGEN ASSAYS IN NORMAL MEN
AGE 24 TO 40 YEARS
(41 assays in 20 men)

Case	I.U.*/24 hr.	No. of Assays	Case	I.U./24 hr.	No. of Assays
1	17	1	11	35	1
2	18	2	12	37	1
3	20	1	13	43	3
4	24	1	14	44	1
5	25	1	15	44	3
6	26	1	16	47	3
7	28	1	17	53	1
8	30	1	18	59	13
9	33	1	19	69	2
10	33	2	20	104	1

* International units.

TABLE 4. URINARY ANDROGENS IN MEN WITH TESTICULAR FAILURE AND MEN WITH ADDISON'S DISEASE, MEASURED BY CAPON COMB GROWTH*

Castrate			Eunuchoidism			Addison's Disease		
Case	I.U./24 hr.	No. of Assays	Case	I.U./24 hr.	No. of Assays	Case	I.U./24 hr.	No. of Assays
21	9	2	29	16.2	5	3	10	1
22	6	2	31	4.4	5	4	10	1
24	12.5	2	32	8.4	5	5	10, 45†	2
26	4	1				6	10	1
27	0	4				9	3	1
						11	7	1
						12	19	1

* Normal range: 18 to 100 I.U./24 hr.
† After two years' treatment with desoxycorticosterone.

gen assays decreased to the same degree. The clinical degree of androgenic deficiency should be clearly distinguished from that indicated by urinary assay.

Prostatic atrophy. The *in vivo* androgenic effects were judged by careful clinical estimation of prostatic size (Table 5). There was consistent and

TABLE 5. DEGREE* OF PROSTATIC ATROPHY IN ADRENAL CORTICAL AND TESTICULAR FAILURE

Castrate		Eunuchoid		Addison's Disease	
Case	Degree	Case	Degree	Case	Degree
21	4+	28	3+	1	0–1+
22	2+	29	4+	2	0
23	4+	30	4+	3	0
24	4+	31	2+	4	3+
25	1+	32	4+	5	1+
26	4+	33	4+	6	0
27	4+			7	3+
				8	2+
				9	0
				10	0
				11	0†
				12	0†
				13	2+

* 0 = normal size; 1+ to 4+ = mild to extreme atrophy.
† Not examined personally.

usually pronounced prostatic atrophy after castration and in eunuchoidism, whereas in men with Addison's disease prostatic atrophy was present only in some cases. In spite of the fact that urinary 17-ketosteroids and androgen levels were low, androgenic effects as judged by prostatic size approximated the normal much more closely in adrenal failure than in testicular failure. However, a tendency to prostatic atrophy in Addison's disease suggests that androgenic effects are decreased in this condition in

TABLE 6. URINARY GONADOTROPIN TITRES IN NORMAL MEN BETWEEN 20 AND 40 YEARS OF AGE

Mouse Units/24 Hours 22 Patients*		Rat Uterine Weight (mg.) 15 Patients	
Mouse Units/24 hrs.	No. of Cases	Uterine Weight (mg.)	No. of Cases
23–26	0	14–20	3
26–53	12	20–40	0
53–105	10	40–60	0
		60–80	7
		80–100	3
		100–120	1

* Ten of the men who had mouse assays had children less than 4½ years of age. All were examined and showed no clinical evidence of endocrine disease.

spite of the presence of intact testes. It should be pointed out that some prostatic atrophy may occur in other chronic debilitating diseases in which adrenal failure may or may not be a factor. The point to which we wish to call attention especially in this respect is that the diminution in androgens in Addison's disease is not connected with high urinary gonadotropins if gonadal function is good. *Cases 6*, *8*, and *10* demonstrate this.

Urinary gonadotropins. Tables 6 and 7 show levels of urinary gonadotropins in normal men and women, according to results of the mouse uterine-weight method. In Table 8 it will be seen that in instances of testicular failure, tests by the rat uterine-weight method show high levels, though this method does not show whether or not they are excessive. They approach the maximum response, but all are exceeded by some of the normals shown. Tests by the mouse uterine-weight method all show levels in excess of the upper limit of normal (105 mg. per twenty-four hours). Table 9 shows high levels of urinary gonadotropins associated with severe oligospermia in men who had no clinical signs of testicular deficiency and had levels of urinary 17-ketosteroids within the normal range (an exception is

seen in one of two assays in *case 33*). This we interpret as due to lack of "inhibin."

Table 8 shows the results of gonadotropin assays in 7 of the 15 men with Addison's disease. The gonadotropins were normal or low in all in-

TABLE 7. URINARY GONADOTROPIN TITRES IN NORMAL WOMEN BETWEEN 19 AND 36 YEARS OF AGE*

Mouse Units/24 hrs. 20 Patients	Age (Yrs.)	Day of Cycle	Length of Cycle, Days
13– 26*	20	14	26
13– 26	21	13	32
13– 26	21	28	29
13– 26	22	23	29
13– 26	22	27	28
13– 26	23	8	24
13– 26*	25	10	?
13– 26	35	18	28
26– 53	30	24	26
26– 53	36	26	27
26– 53	?	16	?
53–105	19	16	28
53–105	20	17	32
53–105	20	20	47
53–105	22	14	?
53–105	25	18	28
53–105	28	18	34
53–105	30	12	28
53–105	?	10	?
53–105*	?	17	?

SUMMARY

Range in M.U.	Patients
13– 26	8
26– 53	3
53–105	9
	20

* All had young children or were shown to have normal vaginal smears except those marked *, in whom smears indicated a mild ovarian deficiency.

stances but one (*case 7*), in which there was absence of one testis and oligospermia. This case shows that a high level of gonadotropins may be present in Addison's disease when testis damage also exists. On the other hand, *cases 6* and *9* indicate that the low levels of 17-ketosteroids and low androgens in Addison's disease were not sufficient in themselves to cause an

TABLE 8. URINARY GONADOTROPINS IN MEN WITH TESTICULAR FAILURE AND MEN WITH ADDISON'S DISEASE

Castrate			Eunuchoid			Addison's Disease			
Case	Rat Uterine Weight* (mg.)	Mouse Units	Case	Rat Uterine Weight* (mg.)	Mouse Units	Case	Rat Uterine Weight* (mg.)	Mouse Units	Sperm Counts (total)
21	91	106–212	28		105+	1		26–53	2 per HPF
22	94	212–424	29		192+	6	20		1165 million
24	89		30		192+	7	80	<105	0
27	High†		31		192–288	8		>6.6	38 million
			32	20					71 million
			33		318+	9		6–13 on 4/12/44	
								13–26 on 6/29/44	
								26–53 on 6/30/44	impotent
						10		>6.6	436 million
						13		6.6–13	

* Rat uterine weight in normal uninjected animals weighing between 30 and 40 Gm. = 17 to 21 mg.

† Friedman test—corpora lutea 3+.

TABLE 9. URINARY GONADOTROPINS AND 17-KETOSTEROIDS IN MEN WITH OLIGOSPERMIA

Case	Rat Assay Uterine Weight (mg.)	Mouse Units	17-Ketosteroids mg./24 hrs.	Sperm Count (HPF)
34	81	480–633	8	occasional
35	82, 74	288–384	11, 13, 10	occasional
36	64	105–212	11, 7	few
37	89, 79	105–212	10	1
38	78	318–424	14	1
39	89, 79, 75	105–212	10	1

elevation in gonadotropins, in definite contradistinction to the effect produced in castration.

Table 10 shows that urinary gonadotropins in women are not elevated in the presence of very low levels of 17-ketosteroids alone (*case 16*) but that when ovarian failure is superimposed, high levels of gonadotropins are produced.

COMMENT

It is interesting that in *case 1* and *case 8* of Addison's disease, prostatic atrophy was present, indicating a lowered androgenic effect with no in-

crease in gonadotropin excretion. In eunuchoidism, if testicular failure is severe enough to produce prostatic atrophy, it would be expected to be severe enough to produce a distinct rise in the excretion of gonadotropins. It is interesting also that in Addison's disease, when mild prostatic atrophy exists in the presence of normal sperm production, no increase in gonadotropins has been found (*case 6*).

In our patients with Addison's disease without evidence of gonadal fail-

TABLE 10. URINARY GONADOTROPINS AND 17-KETOSTEROIDS IN WOMEN WITH ADDISON'S DISEASE

Case	17-Ketosteroids mg./24 hrs.	Gonadotropins (M.U.)	Remarks
14	1	105–212	Age 49. Menopause. Amenorrhea 5 years. Had no hot flashes.
15	1.0	105–212 6–13 in hospital 105–212	Age 35. Ovarian deficiency. Amenorrhea 11 months. Endometrium too scant for biopsy. Vaginal smear deficient.
16	2.0	6–13	Age 33. Menses irregular. Vaginal smear 3+. Premenstrual endometrium showed full progestational response.
17	1.1	318–424	Age 49. Menopause. Last menses two years ago. Hot flashes.
18	1.1	105–212	Age 57. Menopause for 2 years. Vaginal smear similar to that of a castrate.
19	1.5	13–26 13–26	Age 42. Menses regular. Vaginal smear 3+ to 4+.
20	2.1	105–212	Menopause. Hot flashes about age of 35.

ure, no increase in urinary gonadotropins existed in spite of the low 17-ketosteroid levels.

SUMMARY

1. Levels of urinary androgens and 17-ketosteroids found in normal subjects are compared with low levels found in testicular failure and in Addison's disease in men. In the men reported in this paper, urinary 17-ketosteroid levels in Addison's disease and in severe testicular deficiency, approximate half of normal.

2. Judged by prostatic size, androgen deficiency is greater in testicular failure than in Addison's disease.

3. Titres of urinary gonadotropins in normal subjects are compared with those found in testicular failure and oligospermia and in men and women with Addison's disease.

4. Low urinary 17-ketosteroid levels and androgen levels in men with Addison's disease are not associated with high titres of urinary gonadotropins.

5. Severe oligospermia without signs of androgen deficiency and with normal 17-ketosteroids is associated with high excretion levels of gonadotropins.

6. Gonadotropin excretion is normal in Addison's disease (despite loss of adrenal androgens) when gonadal function is normal, and is increased when gonadal damage is superimposed.

7. The data presented are interpreted to mean that while androgen deficiency in men and women has little power to cause pituitary hyperactivity, ovarian deficiency and testicular gametogenic deficiency do have such power. This is considered an additional argument in favor of the existence of a second testicular hormone.

PROTOCOL SUMMARIES

Addison's Disease (Men)

Case 1. Age 33.

Diffuse pigmentation, black freckles, and weakness for fifteen months. Weight loss 23 pounds. A typical crisis had occurred previously. B.P. 114/70 during desoxycorticosterone therapy. Testes of normal size and consistency. Prostate smaller than average. Excellent response to treatment for four years. Died later in crisis.

Case 2. Age 21.

Extreme anorexia and weakness associated with diabetes mellitus. Many black freckles for five months. B.P. 100/70. Water excretion test positive. Great improvement during desoxycorticosterone therapy for four years. Testes and prostate normal. Living.

Case 3. Age 57.

Extreme weakness for four months. Increasing pigmentation. Black moles. Weight loss 10 pounds. B.P. 80 systolic. Water excretion test positive. Response to cortical extract good for two years. Testes and prostate normal. Later died with tuberculosis.

Case 4. Age 29.

Examined in typical adrenal crisis. Deep diffuse pigmentation. B.P. 96/54. Blood chloride 423 mg. per cent. X-ray examination showed pulmonary tuberculosis and adrenal calcification. Response to therapy excellent for four and one-half years. Prostate decidedly atrophic. Testes normal. Died following acute infection.

Case 5. Age 18.

Extremely dark diffuse pigmentation for six years. Extreme weakness for several months. B.P. 72/60. Water excretion test positive. Response to treatment excellent for

five years. Prostate smaller than average. Testes normal. Died following one day of fever.

Case 6. Age 34.

Nausea, vomiting, and weakness for two months. Vitiligo. Black freckles. Dark palmar creases. B.P. 92/78. Water excretion test positive. Working regularly for six and one-half years. Prostate soft and boggy. Testes normal.

Case 7. Age 54.

Weakness, salt craving, and hiccough for four months. Generalized pigmentation and black freckles, especially on pressure areas. B.P. 96/68. Water excretion test positive. Orchidectomy for tuberculous orchitis twelve years previously. Prostate decidedly atrophic. Impotence. Aspermia. Hot flashes. Following treatment with pellets of desoxycorticosterone, improvement was excellent for four years. Living.

Case 8. Age 24.

Increasing pigmentation for one year. Excessive fatigue for two months. Black freckles for one month. B.P. 96/50. Daily fever. Pulmonary tuberculosis evident by x-ray examination. Right adrenal calcified. Prostate moderately atrophic. Improvement during therapy for one year. Drinking bout followed by coma, convulsions, death.

Case 9. Age 39.

Increasing diffuse pigmentation of skin and buccal mucosa for six months. Dark freckles, weakness, hiccough and nausea. B.P. 86/62. Water excretion test positive. Testes and prostate normal size. Response to treatment excellent for three years. Autopsy showed tuberculous adrenals.

Case 10. Age 58.

Weakness for four months. Nausea and pigmentation of skin and gums. B.P. 70/50. Spastic contraction of skeletal muscles. Testis and prostate normal size. X-ray examination disclosed pulmonary tuberculosis. Died in crisis.

Case 11. Age 49.

Weakness, nausea, vomiting, and inconstant indigestion for eight years. Pigmentation of face, hands, and lips for six years. Adrenal crisis diagnosed at Johns Hopkins Hospital. Urine culture positive for tuberculosis. (From notes by Dr. George W. Thorn.)

Case 12. Age 43.

Pigmentation and vitiligo for eight years. Weakness and anorexia for seven years. Repeated crises. Improvement followed treatment with desoxycorticosterone pellets. (From notes by Dr. George W. Thorn.)

Case 13. Age 51.

Incapacitating weakness, anorexia, and indigestion for two months. Loss in weight of 15 pounds. Black freckles. B.P. 80 systolic. Water excretion test positive. Prostate slightly atrophic. Response to therapy good.

ADDISON'S DISEASE (Women)

(For further notes concerning ovarian function see Table 10.)

Case 14. Age 49.

Increasing weakness for one and one-half years. B.P. 80/60. Axillary and pubic hair

very sparse. Wilder test positive. Water excretion test positive. Treatment with desoxycorticosterone pellets, sodium chloride and cortical extract was followed by marked improvement. Amenorrhea for five years. Living.

Case 15. Age 35.

Recurrent attacks of weakness, nausea, and abdominal distress for three years. Diffuse smoky pigmentation of the skin and dark freckles. Axillary and pubic hair very sparse. B.P. 98/68. Water excretion test positive. Treatment with desoxycorticosterone pellets was followed by decided improvement. Associated ovarian failure. Dead.

Case 16. Age 33.

Frequent nausea and vomiting for one year, and diminishing strength. Pigmentation of skin over pressure areas. Typical crisis. B.P. 90/75. Axillary and pubic hair were almost less than normal. Water excretion test positive. Improvement great after desoxycorticosterone pellets and cortical extract. No ovarian failure. Living.

Case 17. Age 49.

Increasing pigmentation of skin for three years. Weakness for two years. Vomiting frequent. Axillary hair absent, pubic hair sparse. B.P. 100/70. Water excretion test positive. Response to cortical extract excellent for two and one-half years. Menopause. Living.

Case 18. Age 57.

Typical pigmentation of skin for two years. Progressive increase in weakness, anorexia, and nausea. B.P. 80/50. Considerable improvement during treatment with cortical extract. Menopause. Living.

Case 19. Age 42.

Weakness, nausea, vomiting, and diarrhea for six weeks. B.P. 72/58. Pigmentation of skin and buccal mucosa. Marked improvement during treatment with desoxycorticosterone implants and aqueous adrenal cortical extract.

Case 20. Age 62.

Increasing pigmentation of skin for two years. Muscular cramps for one year. Pronounced weakness for two months. B.P. 100/70. Excellent response to desoxycorticosterone and salt. Menopause. Living.

Castrate Men

Case 21. Age 66.

History of bilateral orchiectomy for "testicular pain" twelve years ago. Psychoneurosis severe. Physical condition good.

Case 22. Age 52.

Left orchiectomy for trauma six years ago. Right orchiectomy for orchitis. Hot flashes, nervous tension, and impotence controlled by testosterone propionate 25 mg. intramuscularly three times weekly.

Case 23. Age 51.

Bilateral orchiectomy four years previously for carcinoma of prostate. No hot flashes. No evident metastases one year after time of assays.

Case 24. Age 46.

Bilateral orchiectomy for tuberculous orchitis twelve years before assays. Ascending tuberculosis of urinary tract recognized one year before assays. Nutrition good. Nervousness and hot flashes present.

Case 25. Age 69.

Bilateral orchiectomy. Assays one year later during apparent good general health. Impotence and hot flashes present.

Case 26. Age 47.

Bilateral orchiectomy for tuberculous orchitis twelve years before assays. Fatigue and impotence present but general health good. No hot flashes.

Case 27. Age 64.

History of bilateral orchiectomy for suspected testicular tumor. Nervous tension extreme. Frequent hot flashes. Symptoms relieved by testosterone propionate.

Eunuchoid Men

Case 28. Age 41.

Mumps at age 7. Typical eunuchoidism. General appearance and sexual maturity approximately normal for age 15. Testes decidedly atrophic. Sella turcica normal by x-ray examination. Aspermia.

Case 29. Age 19.

No history of orchitis. General and sexual maturity arrested at approximately the 12-year level. Testis biopsy showed tubular hypoplasia, aspermia, numerous Sertoli's cells, rare interstitial cells.

Case 30. Age 37.

Typical severe eunuchoidism. Genitalia of prepuberal proportions. Prostate atrophic. Aspermia.

Case 31. Age 18.

Appearance of moderate immaturity. Penis, testes, and prostate hypoplastic. Aspermia.

Case 32. Age 36.

General skeletal and sexual maturity arrested. Testes partially cryptorchid and prostate aplastic. Aspermia. Sella turcica normal by x-ray examination.

Case 33. Age 32.

Eunuchoidism from testicular atrophy following orchidopexy at the age of 12. Prostate aplastic. Aspermia.

Aspermia and Severe Oligospermia

Case 34. Age 21.

Large bilateral hydrocele. Testes palpable and atrophic. Semen volume 8.1 cc. Rare spermatozoon. Signs suggestive of mild androgen deficiency.

Case 35. Age 28.

Mild eunuchoidism, cryptorchidism, and hypospadias. Prostate half normal size. Rare inactive spermatozoon in semen. Sella turcica normal by x-ray examination.

Case 36. Age 27.

Appearance slightly immature. Left gynecomastia. Penis and prostate normal size. Testes half normal size. Semen volume 2.7 cc. One spermatozoon found. Sella turcica normal by x-ray examination.

Case 37. Age 36.

Genital development and secondary sex characteristics normal, except right testis slightly small. Severe oligospermia. Sella turcica normal by x-ray examination.

Case 38. Age 34.

Mumps at age 10. No recognized orchitis. Genitalia and secondary sex characteristics normal, except left testis small, and prostate one-third normal size. Semen volume 1 cc. Azoospermia.

Case 39. Age 27.

General appearance suggests mild androgen deficiency. Bilateral gynecomastia. Bilateral atrophy of testes. Aspermia on repeated examination. Testis biopsy showed no spermatogenic activity. Interstitial cells appeared normal.

Wassermann and Kahn tests were negative in all patients except in *case 24*, where there was no evidence of active syphilis. In the men with eunuchoidism and aspermia or oligospermia there was no history of venereal disease.

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ANALOGIES BETWEEN URINARY 17-KETOSTEROIDS AND URINARY "ESTROIDS," AS DETERMINED MICROCHEMICALLY*

WILLIAM T. SALTER, M.D., FRANCES D. HUMM, PH.D. AND M. JANE OESTERLING, PH.D.

From the Laboratories of Pharmacology and Toxicology, Yale University School of Medicine, New Haven, Connecticut

SEVERAL years ago Callow and Callow (1) demonstrated that the microchemical determination of 17-ketosteroids could yield valuable information regarding endocrine function in man and other animals. This finding has since been substantiated in many other laboratories (2–6). Nevertheless, considerable opposition to the procedure was raised originally on the grounds that: (a) the urinary 17-ketosteroids constitute a mixture; (b) their value is considerably higher than a biologically equivalent amount of androsterone; and (c) they vary with changes in both testicular and adrenal function. Similar objections can be raised with respect to urinary components related to estradiol.

Degradation products of estradiol in urine

Like testosterone, estradiol is converted within the human organism into degradation products of lower biological potency. The best known of such degradation compounds in the female are estrone and estriol (6). Smith and Smith (7) have also concentrated from human urine a substance which tentatively they regard as a lactone. Furthermore, in animals even after oöphorectomy certain estrogenic effects can be detected, especially in the presence of adrenal hyperplasia (8). Evidence is accumulating that these effects are due to materials which resemble estradiol chemically (9). Under appropriate chemical conditions all of these substances, as isolated from urine, give color reactions similar to estradiol. In the present paper the term "urinary estroids" is used to signify a mixture of steroids which, after extraction from urine and appropriate partial purification, can be estimated microchemically in terms of estradiol equivalents.

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Photometric measurements of estradiol-like compounds

The quantitative study of urinary "estroids" has been delayed for lack of methods sensitive enough to detect the relatively minute amounts present in nonpregnant individuals. In pregnancy, Bachman and Pettit (10) and Stimmel (11) have suggested techniques which are fairly satisfactory for daily excretions of approximately 1 to 3 milligrams daily. Unfortunately, in the absence of pregnancy, the average daily human excretion is less than 100 micrograms even in healthy young women. Friedgood and Garst (12) have applied ultraviolet absorption for this purpose whereas Bates and Cohen (13), Jailer (14) and Finkelstein, Hestrin and Koch (15) have used fluorescence. The data in the present report are based upon a modified Kober's reaction (16). Ordinarily the vermilion shade produced by this phenolsulfonic reagent on reacting with estrone (or estradiol) is obscured by brownish pigments resulting from other urinary chromogens. In the present investigation, therefore, most of these interfering pigments were removed by suitable extraction *after* development of the Kober color.

Simultaneous determination of 17-ketosteroids and of estradiol-like substances

Periodically the problem of androgen:estrogen antagonism appears in the literature (17). The interest in the ratio of these hormones lies in the fact that in certain respects, e.g., in their effect upon the capon's comb, their action is antagonistic or antithetical. The older data were based upon biological assays in which great variability of the test animals combined with great variability among individual subjects to yield widely divergent results. Nevertheless, even under these difficult technical conditions, it was possible to show (18) that an inversion of the ratio of these antithetical substances occurred when men were compared with women. For example, the average woman excreted only half as much mixed androgen as the average man, whereas she excreted at least twice as much mixed estrogen. In the present report the term "antithetical ratio" will be used to signify the arithmetical value derived by dividing the urinary excretion of "estroids" (expressed in micrograms) by the excretion of 17-ketosteroids (expressed in milligrams). This procedure suffers from all the physiologic objections of the older estrogen:androgen ratios *except* that more consistent values are obtained because the variance due to test animals is eliminated.

METHODS

A. For Determination of 17-Ketosteroids. The procedure for 17-ketosteroids was essentially that of Cahen and Salter (4) as applied by

Salter, Cahen and Sappington (19). This method has given results which closely approximate values obtained by Drs. Fuller Albright (20), Lawson Wilkins (21) and Ralph Dorfman (22). *Identical urines* from the patients in their studies were kindly supplied to us for the comparison.

For example:

		mg./24 hrs.		*mg./24 hrs*
Urine #1	Albright	7.8	Salter	7.8
Urine #2	Albright	9.0	Salter	9.1
Urine #3	Dorfman	20.0	Salter	19.9 (Wilkins' patient.)
Urine #4	Wilkins	8.6	Salter	7.5
Urine #5	Wilkins	15.3	Salter	16.6

In any case the procedure gives a consistent range of values in normal healthy young adults. Ordinarily the extract of 17-ketosteroids was made in conjunction with the extraction of estradiol-like substances ("estroids"). Occasionally, however, a small aliquot sample (from 20 to 100 ml.) was extracted separately, as suggested by Drekter, Pearson and McGavack (23). In the latter case however, the colorimetric procedure was that of Cahen and Salter (4) so that all results are strictly comparable.

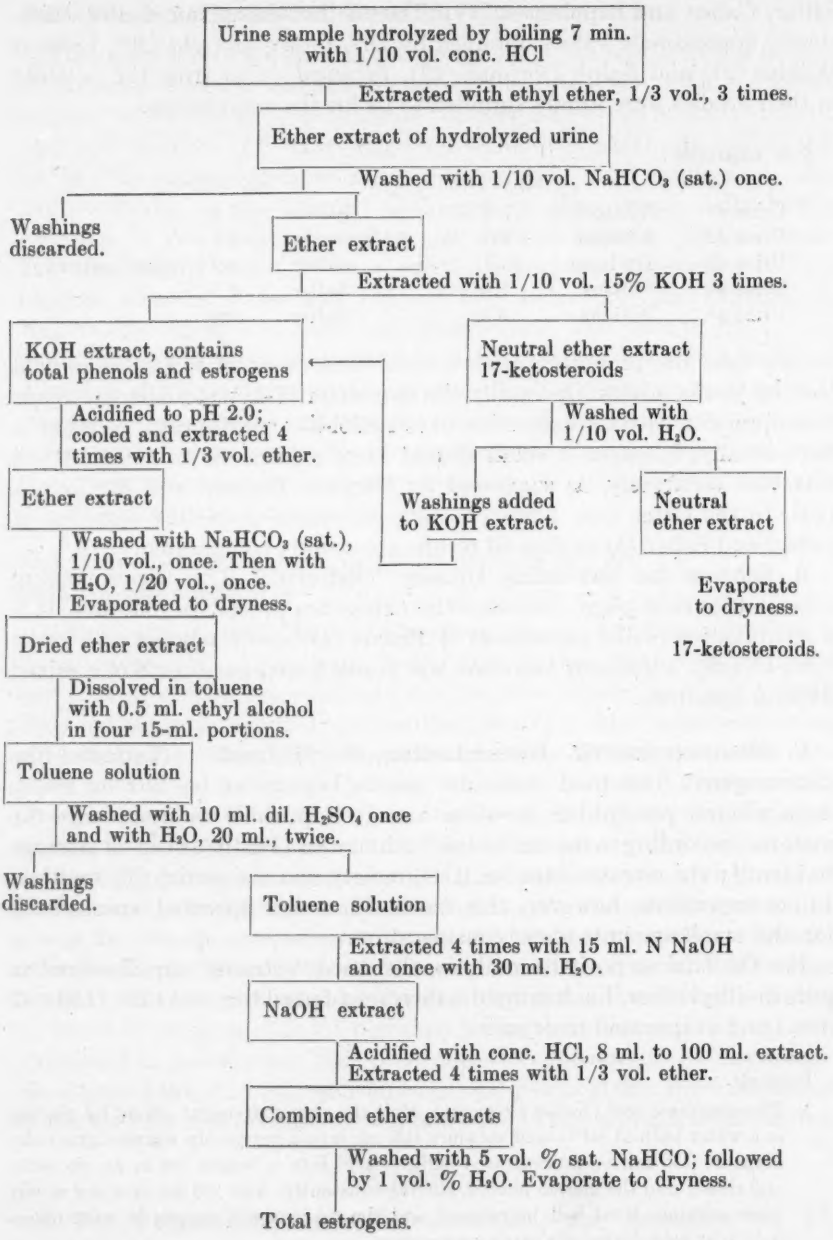
B. Schema for Extracting Urinary "Estroids." The accompanying schema (See next page) indicates the extraction procedure followed. It is a combination of the procedures of Pincus (24) and Bachman and Pettit (10). Usually a six-hour excretion was studied, i.e., one-fourth of a mixed 24-hour specimen.

C. Microcolorimetric Determination of "Estroids" (Estradiol-like Chromogens). The dried "estroids" can be kept in an ice box for many days without perceptible deterioration. It is possible to fractionate the material, according to the method of Bachman and Pettit (10), in an attempt to identify the estrone-estradiol (OD) moiety and the estriol (T) fraction. In our experience, however, this method has not operated successfully for the small amounts under consideration.

For the final step of the analysis, the dried "estroids" are dissolved in pure di-ethyl ether. Each sample is then transferred to a test tube (150×15 mm.) and evaporated to dryness.

Reagents:

1. *Phenolsulfonic acid* (Kober's reagent). Melt the phenol (reagent grade) by placing in a water bath at 60°C. and measure 100 ml. into a previously warmed graduate. Measure 156 ml. of concentrated sulfuric acid into a beaker set in an ice bath, and slowly add the melted phenol, stirring constantly. Add 128 ml. of water slowly while stirring. Heat will be evolved and the reagent will deepen in color unless it is kept cold during the mixing procedure.


Urine sample hydrolyzed by boiling 7 min. with 1/10 vol. conc. HCl
Extracted with ethyl ether, 1/3 vol., 3 times.
Ether extract of hydrolyzed urine
Washed with 1/10 vol. NaHCO₃ (sat.) once.
Washings discarded.
Ether extract
Extracted with 1/10 vol. 15% KOH 3 times.
KOH extract, contains total phenols and estrogens
Neutral ether extract 17-ketosteroids
Acidified to pH 2.0; cooled and extracted 4 times with 1/3 vol. ether.
Washed with 1/10 vol. H₂O.
Ether extract
Washings added to KOH extract.
Neutral ether extract
Washed with NaHCO₃ (sat.), 1/10 vol., once. Then with H₂O, 1/20 vol., once. Evaporated to dryness.
Evaporate to dryness.
17-ketosteroids.
Dried ether extract
Dissolved in toluene with 0.5 ml. ethyl alcohol in four 15-ml. portions.
Toluene solution
Washed with 10 ml. dil. H₂SO₄ once and with H₂O, 20 ml., twice.
Washings discarded.
Toluene solution
Extracted 4 times with 15 ml. N NaOH and once with 30 ml. H₂O.
NaOH extract
Acidified with conc. HCl, 8 ml. to 100 ml. extract. Extracted 4 times with 1/3 vol. ether.
Combined ether extracts
Washed with 5 vol. % sat. NaHCO; followed by 1 vol. % H₂O. Evaporate to dryness.
Total estrogens.

2. *Dilute sulfuric acid.* Add 3 parts by volume of conc. sulfuric acid (C.P., analytical reagent) to 7 parts of water.
3. *Chloroform-ethyl acetate mixture.* Add 2 parts by volume of chloroform (analytical reagent) to 1 part of ethyl acetate (C.P., absolute).
4. *Chloroform-caprylic alcohol mixture.* Mix 25 parts by volume of chloroform (analytical reagent) with 8 parts of n-capry alcohol (C.P., Fisher Scientific Company).
5. *Petroleum ether* (analytical reagent).

Standards:

Estrone, 1 microgram per ml. in 95 per cent alcohol.
Estriol, 1 microgram per ml. in 95 per cent alcohol.

Color Reactions for Determinations of "Estroids"

The following directions indicate the method of developing the color:

1. Into a convenient number, e.g., 12, of clean and dry test tubes (15×100 mm.) place
   a. The reagent blank
   b. Pure estriol (T) standard
   c. Pure estrone (OD) standard
   d, e, f, etc. Unknown samples
2. Dry the tubes in a vacuum desiccator for one hour before the experiment starts. (Meanwhile heat up a bath containing N-butyl phthalate to 130°C. and start the motor stirrer therein. Prepare an ice bath.)
3. After drying the tubes for an hour in the desiccator, remove them and add Kober's reagent as follows:
   a. e.g., if the aliquot of urine is 25 per cent to 35 per cent of a 24-hour excretion, use 1.5 ml. of Kober's reagent and 6 ml. H_2SO_4. Add only Kober's now, H_2SO_4 later, in step 7.
   b. e.g., if the aliquot of urine is 35 per cent to 50 per cent of a day's excretion, use 3 ml. of Kober's reagent and 12 ml. H_2SO_4.
   c. e.g., if the aliquot is less than in "a.", use 0.75 ml. of Kober's reagent and 3 ml. H_2SO_4.

   Always maintain Kober's reagent in proper proportion to H_2SO_4.
4. Heat the tubes at 130°C. for 20 minutes *exactly*. To this end, place the tubes in the bath in series, one every 15 seconds. Agitate the contents of each tube for ten seconds. Remove the series of tubes from the bath in the same order every 15 seconds and stir each tube for ten seconds thereafter.
5. Leave all tubes of the series in ice at least 5 minutes and take all tubes out of the ice together.
6. Allow all tubes to warm to room temperature.
7. Add H_2SO_4 to each tube as specified in step no. 3 above and stir well.
8. From the large tubes pour approximately 3 ml. of each colored mixture into round bottomed test tubes.
9. To each small tube add 1.5 ml. of the ethyl acetate-chloroform mixture. Shake each tube and remove the top layer (containing a dirty precipitate) with a suction pipette.
10. Add 0.5 ml. of petroleum ether. Shake the contents of each tube and remove the dirty supernatant liquor. Most of the dirt comes off at this stage; therefore, draw off as cleanly as possible.

11. Add 1.5 ml. of the capryl alcohol-chloroform mixture, shake, and draw off the top layer. This reagent should be prepared just before using. It contains 8 ml. capryl alcohol and 25 ml. of chloroform.
12. Add 0.5 ml. of petroleum ether and shake. *This time the supernatant layer is not drawn off.*
13. Let each sample stand for one hour at room temperature. Then remove petroleum ether with a suction pipette and transfer to a small colorimeter tube. At least 2 ml. total volume is required in each tube for colorimetric readings.

Colorimetry:

The method of colorimetry involves a green filter (No. 540) and a blue filter (No. 420) as suggested by Stimmel (11). The readings are conveniently converted into corresponding concentrations by the use of a nomogram, pictured in Figure 1. Each investigator should prepare his own nomogram, using known standards, as reported by Oesterling and Salter (25).

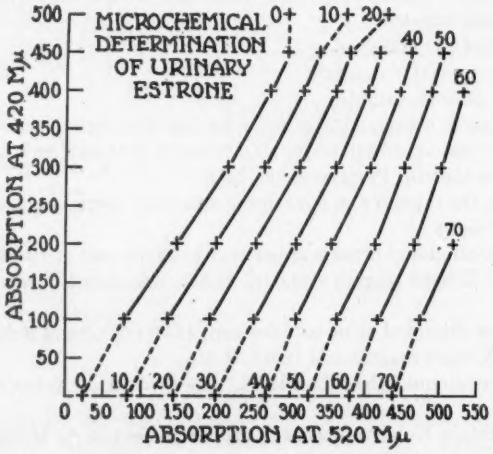


FIG. 1. A nomogram relating photoelectric colorimeter readings to the estrone present.

D. Representative Values for Normal Males. In addition to the normal excretions reported previously from this laboratory (4, 19, 26), in the course of the present investigation data were obtained from normal male students and members of the University staff. These are summarized in Table 1. The average daily 17-ketosteroid excretion from 24 young males was 19.8 ± 3.5 milligrams. The average daily "estroid" excretion from 19 young males was 16.7 ± 9.4 micrograms. The antithetical E/A ratio for 16 young males averaged 0.86 ± 0.49.

E. Representative Values for Normal Females. In the healthy mature female (nonpregnant) the problem of citing normal values is complicated by the dimension of time. The 17-ketosteroid excretion remains remark-

TABLE 1. URINARY DAILY EXCRETION OF 17-KETOSTEROIDS AND "ESTROIDS" IN NORMAL YOUNG ADULT MALES, AS DETERMINED MICROCHEMICALLY

Patient	Age Yrs.	Height	Weight	17-Ketosteroids (hydrolyzed) milligrams	Total "Estroids" (hydrolyzed) micrograms	E/A Ratio
Second Decade		ft. ins.	lbs.			
1. Mr.	19	5 9½	155	13.1	2	0.18*
Third Decade						
1. Hn.	30	5 11½	190	13.4	7	0.49
2. Go.	26	5 11	160	15.7	8	0.51
3. Lw.	22	5 7	135	15.9		
4. Os.	22	5 10½	155	16.2	8	0.48
5. Rg.	21	5 8	145	16.4		
6. Be.	23	5 11	165	17.0		
7. Cm.	21	6 2	155	17.9	23	1.28
8. Br.	22	5 8½	154	18.5	29	1.55
9. Eg.	21	5 9½	165	18.6	26	1.40
10. Sh.	23	5 8	155	18.8	12	0.64
11. Jn.	21	5 9½	140	18.8	17	0.90
12. Bs.	22	6 2½	190	18.9	18	0.96
13. Bt.	25	5 8½	151	18.9	37	1.95
14. Tt.	26	5 8½	150	19.1, 19.1 } 19.1	6	0.31
15. Bn.	23	6 3	180	19.9		
16. Rl.	22	6	165	20.0		
17. Rn.	20	5 10	155	20.2		
18. Js.	20	5 11	150	22.0	11	0.48
19. Sn.	22	5 9	133	23.1, 21.4 } 22.3	27	1.18
20. Nm.	21	5 10	155	22.8	18	0.81
21. Le.	26	5 10	157	25.0	8	0.31
22. Gr.	22	6 1	170	25.1		
23. Pr.	21	6	148	26.2	12	0.47
24. Ce.	23	5 11¾	150	27.1		
				Av. 19.8 ±3.5	Av. 16.7 ±9.4	Av. 0.86 ±0.49
Fourth Decade						
1. Tr.	35	5 7	150	13.8	12	0.88
2. McC.	31	6 3½	190	16.6	17	1.00

* "In the calculation of E/A ratios presented in this report, the arithmetic has been performed *before* the last significant figure of each experimental value has been rounded off. This procedure, which may give the impression of faulty arithmetic, is preferable because it avoids incorporating into the results the slight error of smoothing the last significant figure."

ably constant throughout the menstrual cycle, but the "estroid" excretion varies with ovarian follicular activity. In Figure 2 are presented the findings for both 17-ketosteroids and "estroids" in a normal woman 26 years old.

COMPARATIVE RESULTS

Because the microchemical determination of estrogen-like substances in the urine is now becoming feasible, certain analogies between these

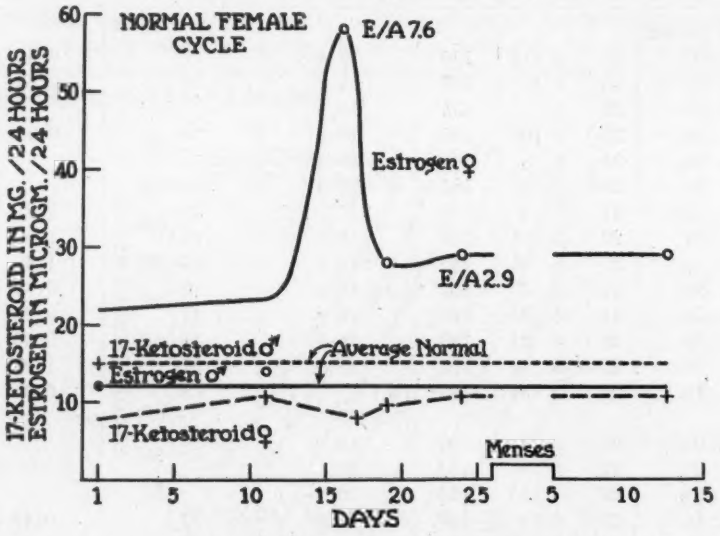


FIG. 2. Variation in urinary "estroids," as determined microchemically, throughout the menstrual cycle of a normal woman, aged 26.

"estroids" and the 17-ketosteroids should be noted at this time, in the hope that useless controversy and futile experimentation may be avoided. It is the purpose of this report to point out five analogies between the respective microchemical estimations of 17-ketosteroids and "estroids."

I. **The effect of age.** It is now clearly established that even in reasonable health, the 17-ketosteroid excretion of mature men declines with advancing years. Representative reports on this point are those of Moore (27) and Hamilton (28). In Figure 3 are illustrated data from the author's laboratory combined with those of Hamilton. In contrast to this trend, the urinary "estroids" tend to increase slightly with age. This second trend is illustrated in Figure 4, in which simultaneous determinations of urinary 17-ketosteroids and "estroids" are pictured for several age groups.

Consequently there is a numerical increase in the antithetical E/A ratio in later life. It might be argued, of course, that this altered ratio is an indication of endocrinological degeneration and therefore is pathological. It seems more practical, however, to establish "normal" standard values for each age group.

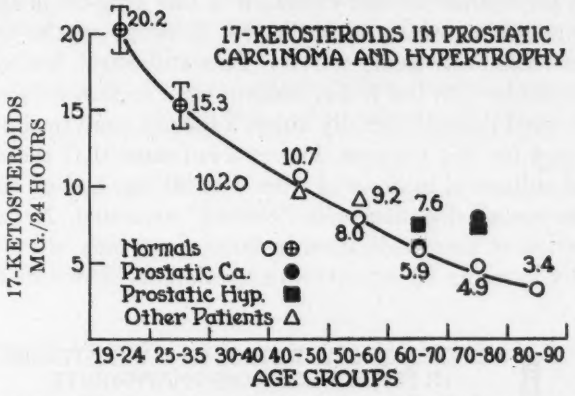


FIG. 3. Effect of age on the daily urinary excretion of 17-ketosteroids in men.

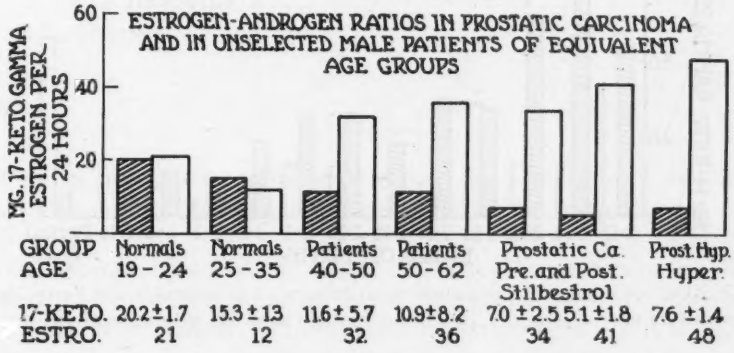

ESTROGEN-ANDROGEN RATIOS IN PROSTATIC CARCINOMA AND IN UNSELECTED MALE PATIENTS OF EQUIVALENT AGE GROUPS
MG. 17-KETO GAMMA ESTROGEN PER 24 HOURS
60
40
20
GROUP Normals Normals Patients Patients Prostatic Ca. Prost. Hyp.
AGE 19-24 25-35 40-50 50-62 Pre. and Post. Stilbestrol Hyper.
17-KETO. 20.2±1.7 15.3±1.3 11.6±5.7 10.9±8.2 7.0±2.5 5.1±1.8 7.6±1.4
ESTRO. 21 12 32 36 34 41 48

FIG. 4. Parallel determinations of urinary 17-ketosteroids and "estroids" for several age groups.

Women in the reproductive age groups present the further complication of the cyclic swing in "estroid" excretion, already illustrated in Figure 2. Near the menstrual period some normal women present absolute values both for 17-ketosteroids and for "estroids" rather similar to the extremes of normal men. Even at this time, however, the antithetical E/A ratio usually remains higher in the female than the arbitrary "upper limit of normality" for males of the same age. A further complication which re-

quires study is the origin of "estroids" found in the castrated or postmenopausal female—possibly the result of adrenocortical activity.

II. **The influence of medication with sex hormones.** Of special interest is the effect of stilbestrol and its derivatives on the urinary "estroids." It has been demonstrated repeatedly (29–31) that large doses of stilbestrol will result in a perceptible urinary excretion of this drug or its esters. Accordingly, after administration of stilbestrol, bioassay of the urine will demonstrate increased estrogenic activity. Pure stilbestrol, however, does not react appreciably with the Kober reagent (16), so that its presence in urine is not recorded microchemically unless a specific analytical procedure (32) is introduced for this purpose. Figure 5 indicates that the daily administration of stilbestrol in doses of from 5 to 20 mg. had no significant effect upon the so-called endogenous "estroid" excretion. Nevertheless, the administration of this medication in large doses may alter the antithetical ratio by lowering 17-ketosteroid excretion, as shown in Figure 5.

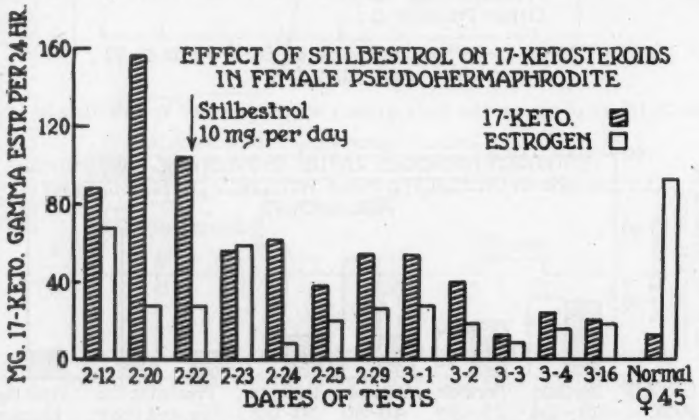


FIG. 5. Effect of stilbestrol medication upon urinary 17-ketosteroids and "estroids," as estimated microchemically, in a female patient aged 42, with adrenal virilism.

The female patient illustrated by the figure (age 42) suffered from generalized hirsutism and an enlarged clitoris, but no adrenal tumor. The internal genitalia were those of the female but the ovaries contained only sparse follicles. As shown in Figure 5, the continuous administration of large doses of stilbestrol caused a definite lowering of the 17-ketosteroid excretion, and thus an increase in the antithetical ratio. The urinary "estroids," however, failed to change significantly. One is reminded of the fact that the administration of methyltestosterone does not increase urinary 17-ketosteroids. Indeed, parenthetically, a slight fall (33) has been reported.

The effect of medication with testosterone, however, is readily detected in a rise of 17-ketosteroid excretion in appropriate cases. For example, a 21-year-old eunuchoid man showed a 17-ketosteroid excretion of only 7.5 mg. per 24 hours. With 150 mg. of testosterone propionate weekly injected intramuscularly, his excretion rose to 17 mg. daily. On the other hand, a 40-year-old woman, two years after oöphorectomy for mammary cancer with metastases, excreted only 4 mg. of 17-ketosteroids daily; and after

TABLE 2. RELATIONSHIP BETWEEN MICROCHEMICAL DETERMINATION OF "ESTROIDS" AND BIOLOGICAL STATUS

Patient	Age Yrs.	"Estroids"(E) micrograms /24 hrs.	17-Keto-steroids (A) mg./24 hrs.	Ratio E÷A
Pseudohermaphrodite				
B	5	129	21	6.1
Simple familial hirsutism				
C.M.	27	30	8.5	3.5
A.C.	28	18	8.3	2.2
H.J.	43	94	7.4	13.0
Adrenal hyperplasia				
M.F.	17	29	63	0.5
S.A.B.	25	39	48	0.8
E.B.	41	29	99	0.3

300 mg. of testosterone propionate weekly, her urinary level rose only to 6 mg. What did the cancerous patient do to the large amount of testosterone?

III. **Adrenal contribution.** That the adrenal cortex contributes to urinary 17-ketosteroid excretion is well recognized (34). Although estrogens have been isolated from adrenocortical tissue (8, 9), it is less well known that such estrogen-like substances may contribute to the urinary "estroids." As indicated in Table 2, however, in a case of true pseudohermaphroditism (kindly referred by Dr. Lawson Wilkins) a remarkably high microchemical value for "estroids" was observed. This precocious child of five years exhibited a urogenital sinus, but no tumor. The abnormal urinary chromogen indicated the presence of a substance with the chemical solubilities of estriol by the procedure of Bachman and Pettit (10) and a low biological estrogenic activity. In the table, the data for this youngster are contrasted with values for older females of two clinical types, i.e., simple hirsutism and adrenal hyperplasia (or metaplasia). The data suggest that

certain clinical syndromes involving hirsutism manifest characteristic antithetical E/A ratios.

Nevertheless, in certain syndromes involving the adrenal cortex both the "estroids" and the 17-ketosteroids may be elevated. If both are elevated to the same degree, the antithetical ratio may be normal.

IV. **Metabolic degradation products.** Among many others, Moore (27) pointed out that the discrepancy between bioassay for urinary androgens and the microchemical value for 17-ketosteroids was due to the low androgenic activity of the several end-products of testosterone metabolism, including their congeners of adrenal origin. According to Gallagher's scheme (6), testosterone is degraded to androsterone, which exhibits only one-third to one-tenth of the biological potency of the precursor. Testosterone also yields etiocholanolone (trans, alpha) and isoandrosterone, the biological potencies of which lie between 1 per cent and 5 per cent of the original testosterone. Equally impotent is dehydroisoandrosterone, presumably of adrenocortical origin. When a mixture of these substances (as extracted from urine) is assayed biologically, the androsterone molecules are weighted more heavily than the etiocholanolone, because the latter has lost most of its potency. Nevertheless, it might well be argued that the inactive molecules are more important because they represent androgenic material which has played its part in bodily metabolism. Indeed a long controversy has been waged over the question whether it is more significant to count "live bullets" or "discharged cartridge shells," to quote a widely used analogy. At least the chemical determination of 17-ketosteroids (4) weights all fractions equally, molecule for molecule, without regard to their final loss of potency.

Much the same problem presents itself for the urinary "estroids" because, as in the case of the androgens, the urinary extracts yield a mixture of partially or completely inactivated hormones. As in the case of the urinary "androgens" also, these metabolic products exhibit a wide range of potencies. In fact, the relative activities of estradiol, estrone and estriol may be regarded as approximately 10:3:1. These values vary widely, however, according to the biological procedures employed. Moreover, the biological behavior of known mixtures cannot always be predicted from the established activities of the individual constituents of such mixtures. A further complication is presented by the potential estrogen described tentatively by Smith and Smith (7) as a lactone. This substance is activated biologically by treatment with zinc and hydrochloric acid. It is interesting that the same treatment increases the microchemical values for urinary "estroids" from twofold to fourfold!

The use of the zinc reduction has two advantages: a) it increases the

total chromogen, thus making for more accurate readings; and b) it salvages certain urines which would be hopelessly contaminated with melanin-like pigment. For example, from 6-hour aliquots of a certain urine the following approximate *relative* amounts of interfering pigment (expressed in color-units) were derived: after simple hydrolysis and extraction, 2000+; after zinc reduction, 1054; after zinc reduction plus clean-up, 268. Inasmuch as the workable range of background is approximately 100 to 400, it is clear that by the use of zinc a result was obtained which otherwise would have been lost.

The advantage of Kober's reagent is that it registers the three urinary estrogens, molecule for molecule, without reflecting any loss of biological

TABLE 3. COLOR DEVELOPED BY PURE ESTROGENS* (at 130°C.)

Time in Minutes	Estrone	Estradiol	Estriol
6	19	23	0, 0, 3
6.5	21, 5, 23	22, 22.5	
7		23	
9	29.5, 31.5	25	7
9.5	33	26	
10			14, 14
10.5	31.5, 32	25, 27	12.5
11	32	27	18, 18
13	33, 33	25, 25	21
15	34	26	26, 26
17	34, 33	23, 26	25
19			37
20	30.5		35, 42, 42
25			40
30			44

* Color units developed by 25 micrograms of estrogen reacting in 4 ml. of Kober's reagent mixture.

potency. This fact was pointed out by Venning (35). In the present investigation, however, it was learned that the time of heating must be regulated carefully lest the estriol fraction develop a disproportionate degree of color. This fact is illustrated in Table 3. It will be observed that in about 20 minutes at 130°C. estradiol and estrone reach maximum color, which is slightly less for estradiol. The color for estriol, however, continues to deepen with time. In the procedure used in this study as little as 2 micrograms of estrone can be detected by the naked eye if interfering chromogens are eliminated.

An example may be cited. In pooled urines from U. S. Army personnel,

TABLE 4. OVER-ALL RECOVERY OF CRYSTALLINE ESTRONE ADDED TO URINE (6-HOUR ALIQUOTS) AND HYDROLYZED AT 100°

Estrone Added micrograms	Estrone Recovered micrograms	Per Cent Recovery
5	5	90
5	4	80
5	3	60
5	2	40
5	2	40
5	2	40
		Av. 58
10	8	80
10	8	75
10	8	75
10	8	75
10	6	60
10	6	60
10	6	60
10	4	40
10	4	40
10	3	30
10	2	20
10	2	20
10	0	0
		Av. 49
20	17	85
20	12	57
20	10	47
20	9	45
20	9	45
20	9	45
20	9	42
20	8	40
20	5	25
20	5	22
20	5	25
		Av. 44
30	15	50
50	21	42
		Av. 46
Grand Average		49%

the authors found a daily excretion of "estroids" corresponding chemically to approximately 11 micrograms of estradiol. By a modified Thayer-Doisey (36) bioassay procedure, however, the result was only 3 micrograms. Moreover, in gynecomastia based on malnutrition (26) the authors found even lower values by both methods; but the antithetical E/A ratio was normal. In gynecomastia, it seemed important to exclude the possibility that large amounts of highly *inactivated* estrogen were present in the urine. Therefore the microchemical procedure seemed particularly important in this latter instance, because it revealed a low "estroid" excretion in the presence of abnormal mammary hyperplasia.

V. **Technical problems in methodology.**

17-KETOSTEROIDS: In the microchemical determination of the urinary 17-ketosteroids, two major technical difficulties plague the investigator. These are:

a. the quantitative recovery of the 17-ketosteroids in a partially purified state; and

b. the elimination of extraneous pigments or interfering "background" during the final colorimetric procedure.

In the case of the 17-ketosteroids one widely used chemical method (20) is based on an optical correction for "background," whereas another method (4) involves the removal by extraction of the extraneous pigments. Both

TABLE 5. RECOVERY OF CRYSTALLINE ESTRONE FROM CLEAN-UP PROCEDURE

Estrone Added micrograms	Estrone Recovered micrograms	Per Cent Recovery
10	12	120
10	10	100
10	8	80
10	7.5	75
10	3.5	35
15	5.5	33
20	24	120
20	13	65
20	8	40
30	38	126
30	30	100
Grand Average		81%

of these obstacles are present in the microchemical determination of urinary "estroids," and both devices have been used to reduce the interfering pigments.

"ESTROIDS": *a. Quantitative extraction.* With respect to the recovery of urinary "estroids," the problem is essentially the same as in bioassay. In both procedures it is necessary to hydrolyze the conjugated estrogens and to separate them from a large mass of undesirable material. Under routine laboratory circumstances the recovery of added estrogen (i.e., a female complement introduced into male urine) may approximate 50 per cent. In Table 4 are given a series of recoveries from male urine "spiked" with

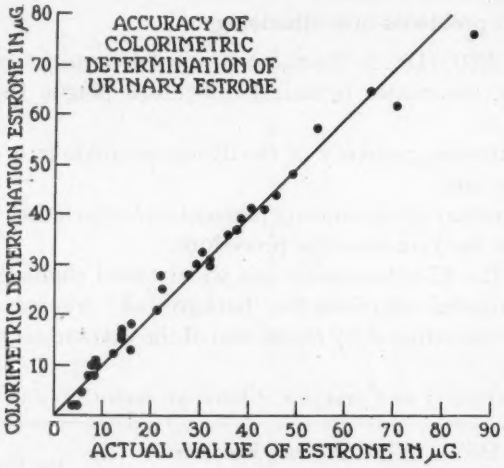


FIG. 6. Comparison of observed and theoretical values obtained by means of the nomogram shown in Figure 1.

known amounts of estrone. It should be noted that the over-all recovery of approximately 50 per cent can be broken down according to the individual stages of the procedure as follows:

1). Initial hydrolysis and extraction, separation and concentration of "estroids," 39 per cent loss.

2). Clean-up procedure to remove interfering brown pigment, 11 per cent loss. This loss is shown in Table 5.

3). Final colorimetry, 0 ± 3 per cent loss.

To those scientists who refuse to think in terms of a sliding scale when dealing with microanalysis this poor recovery will seem discouraging. It is clear at present that only relative values can be determined for urinary "estroids," whether bioassay or a microchemical procedure is used. Nevertheless, as a practical aid in improving extraction procedures, the colori-

metric estimation has distinct advantages over bioassay, in both accuracy and the amount of labor involved. The accuracy of the final colorimetric procedure is illustrated in Figure 6.

b. Elimination of interfering pigments. The amount of interfering background pigment varies widely from day to day in the same human subject. Indeed, an occasional urine specimen is encountered which is hopelessly "contaminated" with undesirable background pigment, and must therefore be discarded. Possibly a rigid control of diet and medication might reduce this unwanted chromogen to low values. The extraction procedure based on differential polarity removes much black tar and often over 90

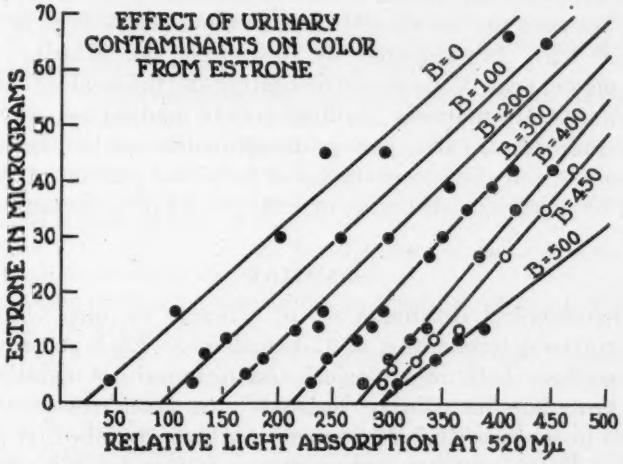


FIG. 7. The relation of extraneous "background" pigment to the photoelectric estimation of estrone. Each line represents a certain concentration of "background" as measured through a blue filter (No. 420).

per cent of the dark interfering pigment. Even under the most favorable circumstances, however, it has not been possible to extract completely the brownish color without a serious loss of the true vermilion of the estradiol-like chromogens.

Therefore, after the bulk of this material is gone, an optical correction is requisite, as shown in Figure 7. This correction may be applied algebraically at considerable cost of time and labor. It is more conveniently incorporated in a nomogram, such as that shown in Figure 1. This chart is based upon the tacit assumption that the composition of the undesirable pigment is invariable. As in the case of 17-ketosteroids, this assumption is not strictly true. Indeed, the method of Cahen and Salter (4) for 17-ketosteroids was developed in order to side-step this difficulty. In the case of the

"estroids," however, the assumption must be faced at present. Although it doubtless introduces some variability into the data, there is no evidence from recovery experiments that average figures are altered appreciably by the optical correction. In short, until the recoveries of estroids from crude urine are much better than at present, the present optical technique is adequate (although unsatisfactory).

Of course if a sensitive spectrophotometer is available, more accurate results can be obtained and the nomogram can be dispensed with. The absorption curve of the "background pigment" will vary somewhat with the extraction and clean-up procedures used. A suitable study of its optical behavior, however, will reveal a wave band which will accentuate the difference between the brown and the vermilion colors (e.g., at approximately 520 mμ). As suggested by Stevenson and Marrian (37), the vermilion pigment can be destroyed by heating and the residual background read afterwards. Alternatively, readings may be made at two wave lengths (e.g., 430 and 520 mμ, after *precise* determination under the individual circumstances), and the concentration of vermilion pigment may then be estimated by algebraic calculation or with the aid of a nomogram.

SUMMARY

The microchemical determination of urinary "estroids" bears many analogies to the determination of 17-ketosteroids. Each procedure raises similar questions, both physiological and technical. A tentative microchemical procedure for urinary "estroids" has been mentioned and its application in selected clinical instances has been described. It is emphasized that at the present time only relative significance can be expected of the values so obtained. Many obstacles militate against absolute accuracy for "estroid" excretion, but similar obstacles exist for the estimation of 17-ketosteroids.

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ALKALINE PHOSPHATASE AND GLYCOGEN IN HUMAN ENDOMETRIUM

J. P. ARZAC, M.D. AND E. BLANCHET, PH.D.

Laboratory of Endocrine Gynecology of Hospital Español, Mexico, D. F.

BECAUSE of the morphogenetic properties of the sex hormones, the histologic study of accessible sex organs has been demonstrated to be very useful in the evaluation of genital functions. However, we believe that the histochemical study of such organs and tissues promises a better understanding of the many problems involved.

So far as we know, glycogen and lipids are the only substances which have been systematically investigated by means of histochemical reactions during the human endometrial cycle. Except for the excellent work by Wislocki and Dempsey (1) on the mucosa of the pregnant uterus, phosphatases have not been studied in the human endometrium. It can be stated that, to date, only observations on the *location* of phosphatase have appeared in the literature, such as the report of Kabat and Furth (2), who demonstrated the absence of phosphatase in the myometrium and its presence in small amounts in the epithelia. The relation between this enzyme and estrogen action in mouse endometrium has been shown by Atkinson and Elftman (3) and mentioned by Gomori (4, 5).

The purpose of this paper is to report our findings on this subject and to indicate some problems that arise from the possible relationship between alkaline phosphatase and glycogen. It is well known that a mechanism of phosphorylation catalyzed by phosphatases exists in the metabolism of glucides and lipids (Dempsey and Wislocki (6)), which in all probability plays an important role in the nidation of the ovum and the endocrine functions related to the endometrium.

METHODS

The endometrial specimens obtained by biopsy were immediately fixed in 96 per cent alcohol and often refixed in alcohol-formol-picric mixtures (unpublished data). For the study of glycogen, this method of fixation has proved to be very helpful when carmine dyes are used.

Carmine, the leuco-fuchsin reaction for aldehydes, and the silver methods devised by Mitchell and Wislocki (7), Gomori (8), and one of us (9), have been used for the demonstration of glycogen. Digestion in saliva and adequate pretreatments were employed in order to rule out other substances. Because of the advantages of the silver procedures, these have been favored in the present investigation.

For the demonstration of alkaline phosphatase, Gomori's technic (4)

as modified by Wachstein (10) has been followed. Some slight variants however have been introduced in certain cases; as for example, shortening of the incubation period under higher temperatures. Some functional differences could be shown by this means, such as the frequent presence of the enzyme in the stroma, near the glands and capillaries, and its presence in extensive cytoplasmic zones that otherwise would have been ignored. Hematoxylin-eosin was used for the "control" slides. Counterstaining of glycogen and phosphatase reactions was generally omitted. Most of the time, both glycogen and phosphatase reactions were done on each specimen.

RESULTS

Four categories (two of them subdivided in 3 groups each), as judged by the morphological patterns and the clinical data available, have been composed from 73 specimens studied. The following key was used for the interpretation of the reactions obtained.

Alkaline Phosphatase

0	Negative reaction
+	Positive reaction in capillaries only
++	Positive reaction in nucleoli also
+++	Positive reaction in nuclei
++++	Positive reaction in cytoplasm

Glycogen

0	Negative reaction
+	Very small granules
++	Coarse granules
+++	Small masses
++++	Large amounts

Although arbitrary and relative, the above scale made possible a quantitative estimation of the histochemical reactions obtained. This is shown in Table 1 for 68 specimens. Three specimens of decidua and two showing endometrial tuberculosis were also studied.

Since most of the samples came from gynecological patients, abnormalities prevailed in the whole group. Moreover, a certain selection was made in order to secure several morphological types. A brief description of our findings follows:

A. The distribution of alkaline phosphatase

During the estrogenic phase epithelia and capillaries are rich in phosphatases and this seems to increase in proportion to the estrogen stimulus (see Table 1). Gland cells and epithelial surface cells show the enzyme in all their structure, both nuclei and cytoplasm (Fig. 1). As the cycle advances, particularly from the midcycle on, the cytoplasm and the nuclei show a less intense reaction. Thus during the transitional phase, although the

enzyme is still abundant, especially in the epithelial surface, subnuclear vacuoles appear. The nuclei give a positive reaction mainly in the nucleoli and very often just the apical portion of the cytoplasm shows an intense reaction. This is also shown by secretion in the gland lumen. The epithelial surface remains unchanged for a longer period of time. During the progestational phase only secretions, capillaries and sometimes nucleoli produce a positive reaction (Fig. 2); still later, near menstruation, only the capillaries. Practically no reaction is present during menstruation although some variations exist in different specimens. The reaction in the stroma is almost always negative and only during the progestational phase have we seen positive reactions in the nucleoli and certain decidua-like periglandular and perivascular cells.

TABLE 1. RESULTS OF REACTIONS FOR ALKALINE PHOSPHATASE AND GLYCOGEN IN HUMAN ENDOMETRIUM*

ENDOMETRIAL TYPE	ALKALINE PHOSPHATASE		GLYCOGEN	
	No. of cases	Average†	Average†	No. of cases
ESTROGENIC				
Hypoestrogenic	9	0/+ (0.44)	+/++ (1.7)	9
Normal	4	+++/++++ (3.5)	+/++ (1.5)	4
Hyperestrogenic	5	+++/++++ (3.6)	+/++ (1.8)	6
TRANSITIONAL	6	++/+++ (2.4)	++/+++ (2.2)	5
PROGESTATIONAL				
Hypoprogestational	20	+/++ (1.6)	++ (2.1)	19
Normal	10	0/+ (0.66)	+++/++++ (3.2)	9
Menstrual	8	0/+ (0.62)	+/++ (1.6)	8
IN THYROID DISEASE	6	++++ (4)	+/++ (1.6)	5
Total	68			65

* For key, see section on "Results."

† The figures in parentheses are an attempt to express quantitatively the averages of the reactions obtained for each group.

The main characteristics in the definitely abnormal cases are irregularities of distribution and increase, decrease, or even absence of phosphatase paralleling the degree of endometrial development. An important exception was found in the somewhat paradoxical picture of the endometrium in hypothyroidism (11, 12), which we hope to discuss in a separate paper.

Up to the present time only three deciduas have been studied. In two cases of spontaneously eliminated fragments we found negative reactions. This disagreement with the findings of Wislocki and Dempsey (1) can be explained, we believe, by the endocrine abnormalities of the patients, who showed a tendency toward abortion and bleeding.

The third specimen unexpectedly obtained by biopsy was very rich in phosphatase. The almost absolute absence of glycogen in all three specimens may support this hypothesis.

In two cases of endometrial tuberculosis we did not find any enzymatic activity in the giant cells.

B. The distribution of glycogen

The amount of glycogen present in the endometrium during the estrogenic phase varies from traces to coarse granules situated basally or surrounding the nuclei of the

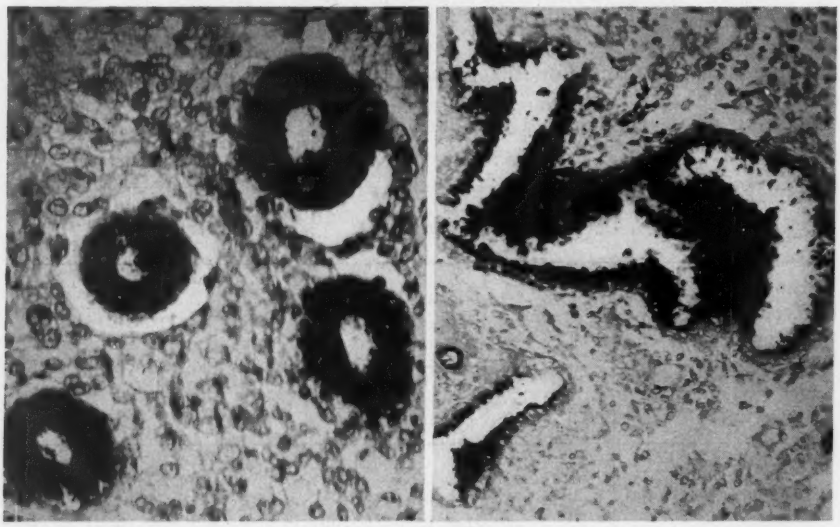

FIG. 1. Alkaline phosphatase during the estrogenic phase (×350).

FIG. 2. Capillaries and secretion are positive for alkaline phosphatase during the progestational phase (×250).

glandular and epithelial cells (Fig. 3). It is even possible to see such granules in the process of being secreted. During the transitional phase, morphologically considered as the ovulatory phase (although we believe it is not necessarily so), the subnuclear and often all the perinuclear zones have been found loaded with glycogen. Later on, as the progestational phase advances, the epithelial cells, especially the gland cells, discharge into the apical portion of the cytoplasm or into the gland lumen. The stroma shows more and more granules and cytoplasmic masses of glycogen. During menstruation the polysaccharide seems to be washed out and the amount diminished. In some instances a considerable quantity was found in the vascular walls.

Specimens poorly transformed or irregularly matured by progesterone showed scanty and unevenly distributed glycogen tending to be confined inside the gland cells as described by Hughes (13). On the other hand, in one case (not tabulated) from a luteal ovarian cyst there was a large amount of glycogen in the numerous glands present. A fairly large quantity was contained in hyperplastic endometrium (Fig. 4) without morphological signs of progestational response. In Figure 5 is seen a curve which schematically

expresses different levels of both substances during a more or less theoretical cycle[1] and during some of its hormonal abnormalities.

DISCUSSION

We believe that the results obtained are fairly consistent. With respect to our findings in the investigation of human endometrial glycogen they coincide in general with the data reported by others (13, 16, 17, 18, 19, 20).

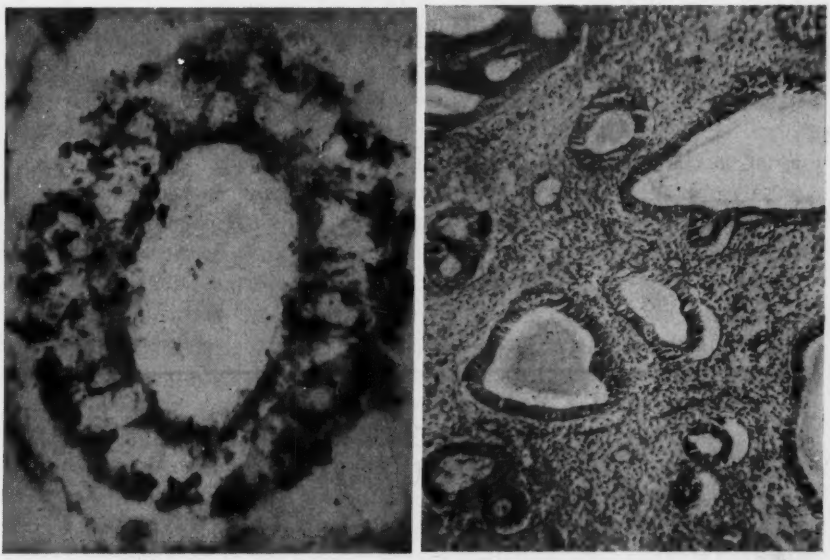

FIG. 3. Glycogen in a morphologically estrogenic specimen (Arzac's silver method) (×720).

FIG. 4. Cystic hyperplastic endometrium showing glycogen granules and small masses basally and around the nuclei (Arzac's silver method) (×100).

[1] We have said a "more or less theoretical cycle" because it is well known from biologic determinations that the hormonal levels of estrogen and progesterone during the *real* cycle oscillate in a way essentially similar to the one expressed in the abscissa of our graph (excepting the HE point). The estrogen curves of D'Amour (14) and of Markee and Berg (15) for example show a relative hypoestrogenic status at the beginning compared to more advanced epochs of the cycle, near ovulation time. Furthermore the points hE and hL of our graph correspond to reactions observed in specimens taken in the early proliferative and early secretory phases. In these, the histological aspects correspond to about the seventh to the ninth days and the twentieth to the twenty-second days of the normal cycle, respectively. On the other hand, the numbers in the ordinate are not strictly subjective values but an attempt to express quantitative averages (see Table 1).

The premenstrual peak is particularly pronounced, whereas other peaks are not quite so definite. However, some facts often forgotten or debated (Moricard (21), Beclere (22)) deserve to be emphasized, namely, the existence of rather large amounts of glycogen in purely estrogenic endometria (Fig. 3), showing either morphologic hypoplasia or glandular cystic hyperplasia (Fig. 4). Of course it is not unusual to obtain positive reactions

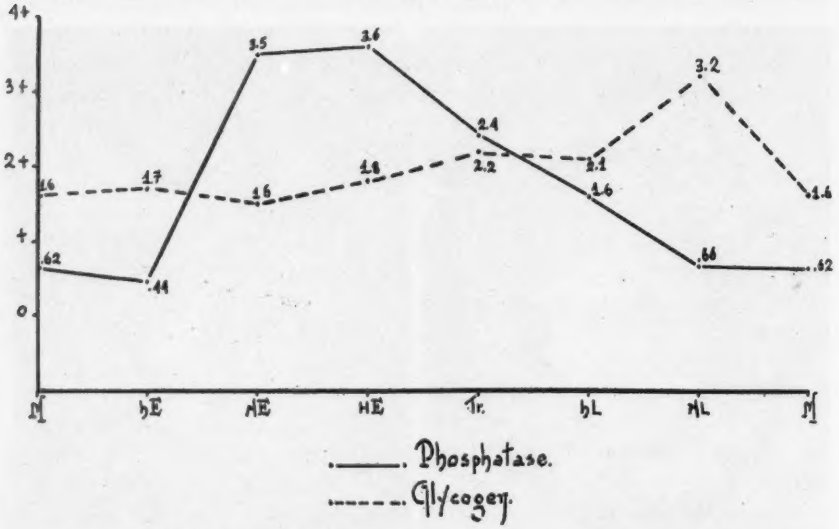

FIG. 5. Graph composed of the averages of the estimated values for alkaline phosphatase and glycogen obtained from 68 specimens (see Table 1). M (menstruation); hE (hypoestrogenic); NE (normal estrogenic); HE (hyperestrogenic); Tr (transitional); hL (hypoluteinic); NL (normal luteinic).

in "mixed" specimens with or without hyperplasia. It seems important, however, to stress the fact that in some cases (such as those which show a hypoestrogenic condition morphologically) estrogen alone seems sufficient to stimulate the appearance in and even a slight discharge of glycogen from the glands of the human uterine mucosa. In this respect the situation seems to be identical with the one in macacus rhesus (Hisaw (16); Overholser and Nelson (23)) and in the rat (Boettiger (24)). This has escaped the attention of many gynecologists because of a lack in the past of histochemical procedures as efficient as the silver techniques previously mentioned. In fact Zondek and Stein (20), and Spyker and Fidler (19) were able to demonstrate glycogen during the estrogenic phase by chemical analysis but not histochemically.

From another point of view our results are in disagreement with the

findings of others. Thus Spyker and Fidler (19) did not find glycogen in the stroma whereas in our specimens we were able to demonstrate considerable amounts of it. Perhaps the explanation advanced for similar findings by Hughes (13) would apply in this instance, as many of our specimens came from cases of sterility. Nevertheless, it must be pointed out that most of our morphologically normal progestational specimens revealed glycogen in the stroma. It is interesting also that morphologically pure estrogenic specimens, even those premenstrual biopsy specimens showing hypoplasia, had somewhat larger amounts of glycogen than those from the proliferative phase of normal cycles. A lack of parallelism between morphology and chemical status in such instances may perhaps be more frequent than is thought.

Concerning phosphatase it seems quite clear that progesterone has an effect upon this endometrial enzyme not established, so far as we know, by previous investigators. Thus the only mention that we have found in current literature is the one made by Atkinson and Elftman (25) who state that the circular muscle layer of mouse uterus shows a disappearance of the reaction when the animal is treated with progesterone and that the results elicited by estrogen and progesterone are the same as after estrogen alone. Our data show on the contrary that, in human beings at least, the responses are quite different from each other and that the more advanced the progestational phase, the smaller the amount of phosphatase present in the endometrium; whereas the opposite happens during the estrogenic phase.[2] This cannot be attributed to a lack of estrogenic action during the luteal phase since it is well known that the quantity of this hormone increases during the second half of the cycle.

The relationship between alkaline phosphatase and glycogen is very interesting. It seems quite clear that the amount of each, particularly during the progestational response, shows an inverse ratio. This suggests that glycogen is formed at the expense of phosphatase, which is in agreement with the phosphorylation theory of glycogenesis. In fact Wislocki and Dempsey (1) have reported that whenever a process of glycogen deposition is found, a barrier or a bed of phosphatase is always present or interposed between circulating blood and the place in the tissue where glycogen is deposited. Since only the endometrium of pregnant women was studied by them, they reported the presence of phosphatase only in the capillaries (at the beginning of pregnancy) and they supposed that this barrier was sufficient explanation for the appearance of the polysaccharide

[2] After this paper was written our attention was called to a very recent publication by Atkinson and Engle (26) who, in a smaller number of cases, found essentially the same results as ours in monkey and human endometrium.

in different places in the mucosa. Our results show however that in human endometrium a bed, in addition to a barrier, of phosphatase is found sometime during the cycle, preceding the deposition of glycogen in the particular cells involved.

If we consider now what happens under the influence of estrogenic action it is evident that glycogen, although in small amounts, is synthesized in the presence of large amounts of the enzyme; and that in certain cases (during the late estrogenic and the transitional phase) both are present in rather large quantities. This suggests that on the one hand the presence of sugar and phosphatase is not sufficient for the establishment of a full catalytic process of polymerization; and on the other hand, that the whole process is gradually and not suddenly established.[3]

It appears safe to assume that progesterone is the factor involved in the activation of the catalytic reaction, evident during the progestational phase. Since, however, this reaction, although less intense, can be evoked under the influence of estrogen alone, several hypotheses can be advanced tentatively: (a) estrogen and progesterone have, from this particular point of view, only a quantitative and not a qualitative difference; (b) progesterone is secreted during other than the second half of the cycle; (c) metabolites, progressively produced under estrogenic action, progressively activate the catalytic reaction; (d) several enzymes act at different epochs of the cycle. For the time being we have no conclusive evidence to support any one of the mentioned hypotheses to exclusion of the others, so that it is more than likely that several factors are involved in the production of the phenomena described. Of course the role of phosphatase in the process of growth cannot be excluded, since it seems to be involved in the synthesis of nucleoproteins. In fact, we found a very intense reaction, together with the presence of vitamin C granules, in such an actively growing tissue as a highly malignant endometrial adenocarcinoma (not tabulated). On the other hand, the part phosphatase can play as a respiratory enzyme is another possibility. In this respect the observation that a maximum response is found in the endometrium of those patients in whom thyroid function is subnormal, deserves careful consideration.

It is obvious that routine studies of the two histochemical reactions here mentioned are highly desirable since they can throw a great deal of light on the physiology of the uterine mucosa. This may be particularly true in cases of sterility which show a definite tendency toward phosphatase-glycogen imbalances, either because the enzyme appears increased due to poor utilization for glycogen synthesis, or because both are dimin-

[3] The facts just reported by Zondek and Hestrin (27) give support to this point of view.

ished when glycogen synthesis is decreased due to scarcity of the enzyme. Perhaps anovulatory menstruation and other "non-menstrual" bleedings will be differentiated more clearly by such studies.

SUMMARY

1. Alkaline phosphatase and glycogen have been studied histochemically in seventy-three biopsies of human endometrium.

2. It was found that the amount of alkaline phosphatase demonstrable in the endometrium increases during the proliferative estrogen phase of the menstrual cycle, whereas it decreases during the progestational phase and disappears during menstruation. It is abundant in the endometria of patients with gynecological disorders associated with hypothyroidism.

3. With the silver techniques used, glycogen could be histochemically demonstrated not only during the progestational phase but also during the proliferative phase; and in hypoplastic as well as in hyperplastic estrogenic specimens. The results with these procedures agree in a general way with the quantitative biochemical data so far reported in the literature available to us.

4. The results obtained are discussed and the suggestion made that the histochemical study of the endometrium is highly desirable, particularly in cases of sterility.

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USE OF GLOBIN INSULIN IN ADDISON'S DISEASE ASSOCIATED WITH INSULIN-SENSITIVE DIABETES

W. L. LOWRIE, M.D., W. E. REDFERN, M.D. AND D. P. FOSTER, M.D.

From the Metabolic Division, Department of Medicine, Henry Ford Hospital, Detroit 2, Michigan

IN 16 cases of coexisting diabetes mellitus and Addison's disease so far reported in the literature, insulin sensitivity has been a frequent occurrence and has presented a difficult therapeutic problem. In three of the cases reported, diabetes developed after the onset of Addison's disease (1, 2, 3).

Simpson's (1) patient had been treated elsewhere with epinephrin injections. Following treatment with adrenal cortical extract, hyperglycemia and glycosuria developed within one month. Insulin was administered. This resulted in severe hypoglycemic reactions from even small doses. The patient eventually came to autopsy and the diagnosis of Addison's disease was substantiated.

The patient described by Rhind and Wilson (3) was treated with desoxycorticosterone for one year and then developed diabetes. This individual was the only one of fifteen who was apparently not extremely sensitive to insulin. The patient was taking 60 units of insulin per day and eventually expired in a hypoglycemic convulsion.

Thorn and Clinton (2) reported a case of Addison's disease treated with desoxycorticosterone for three years prior to the onset of diabetes. Small doses of regular insulin were given. By this method, severe reactions, such as might be induced by protamine zinc insulin, were avoided.

In the remaining thirteen cases which were reported, the Addison's disease developed either concurrently with diabetes (4) or after the diabetes (4–13).

Rogoff (14) reported a patient, who, before coming under his care, had had the adrenals denervated in order to alleviate diabetes. The diabetes, prior to operation, had been controlled with diet and insulin. The patient developed Addison's disease. His insulin requirement decreased and he became sensitive to insulin. The procedure was condemned by the author.

Armstrong (15) reported a patient who had diabetes associated with Addison's disease and whose insulin requirement prior to the development of Addison's disease was 56 units. After development of Addison's disease his requirement dropped to 8 units daily. The patient was sensitive to

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insulin and developed hypoglycemic reactions. In this case adrenal cortex extract (Eschatin) produced glycosuria and hyperglycemia.

CASE HISTORY

In our patient, a colored female, aged 30, Addison's disease was diagnosed on her first admission, December 7, 1944. Complaints: loss of energy and weight, anorexia, nausea and diarrhea. She also stated that her skin had become darker and that pigmented areas had appeared on her gums. She was extremely emaciated and asthenic. Her blood pressure was 70/40. Blood chemistry showed serum sodium, 126.9 mEq./L, and potassium, 4.75 mEq./L. Two days later the potassium was 5.1 mEq./L. Glucose was 66 mg. per 100 cc. and CO_2 combining power, 20.4 mEq./L. Hemoglobin was 14.6 Gm., red blood cells 4.46 million and white blood cells 7,200. The differential count was normal. The water excretion test (16) showed the following: part I, positive; part II, 4.9 (normal 25+).

The Addison's disease was brought under control by glucose, sodium chloride, adrenal cortical hormone and desoxycorticosterone. The natural hormone was continued for seventeen days and a total of 170 cc. (425 rat units) was given. The symptoms were relieved. The patient gained strength and her weight varied from 95 pounds to 103 pounds.

At first, 15 mg. of desoxycorticosterone acetate were required daily but at the end of nine months she was able to do light housework while taking 5 mg. every other day. At this time three 75 mg. pellets of desoxycorticosterone[1] were implanted beneath the skin. The patient's capacity for work remained good for a period of six months, after which she reentered the hospital complaining of weight loss (7 pounds), polyuria, excessive thirst, anorexia, and weakness.

Physical examination: Temperature 99°, pulse 74, respirations 20, blood pressure 70/50. Examination revealed an emaciated colored female with evidence of dehydration. Skin folds along the thighs and legs showed deeper areas of pigmentation. Pigmented areas were also present in the mucous membrane of the gums. Sibilant râles were heard in the upper lobe of the right lung, posteriorly. There were no significant findings in the heart or abdomen. There was no Babinski. Pelvic and rectal examinations were negative.

The first impression that the patient's condition was due entirely to undertreatment of the adrenal cortical insufficiency, was not substantiated by the laboratory findings.

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[1] Desoxycorticosterone pellets were supplied through the courtesy of Dr. Edward Henderson, Schering Corporation, Bloomfield, New Jersey.

Blood:

Serum sodium	305 mg. per 100 cc.; (132.6 mEq./L)
NPN	32 mg. per 100 cc.
Glucose (fasting)	346 mg. per 100 cc.
CO_2	47.5 per 100 cc. (21.3 mEq/L)
HB	12.4 Gm. per 100 cc.
RBC	3.98 million
WBC	7100

Cephalin-cholesterol liver function test—negative.
Thymol turbidity—1 unit.
Thymol flocculation—0.
Urinalysis—S.G. 1,020; albumin negative; glucose 4 plus; acetone positive; diacetic acid negative.
Skin test for hemochromatosis (17)—negative.
24-hour urine for hemosiderin—negative.

The patient's condition was not improved by supplementary desoxycorticosterone, 5 mg. daily, or by increased sodium chloride intake. Weight did increase after insulin was started. However, hypoglycemic reactions of unusual severity occurred without warning even from as small a dose as 5 units of regular insulin. The reactions were not always accompanied by perspiration. On several occasions the patient was found comatose in bed and unable to swallow. In each instance the onset of hypoglycemia was so rapid that disorientation or loss of consciousness developed before the patient could summon help although she was in a three-bed ward. In spite of the reactions, her general condition improved. She gained weight and strength. On March 13, 1947, her temperature rose to 102° and there was evidence of bronchopneumonia. Penicillin, 25,000 units every three hours, was given and the temperature gradually fell to normal within four days (Fig. 1).

On March 31, 1947, two 75 mg. pellets of desoxycorticosterone were implanted and intramuscular injections were discontinued. In view of the severity of the insulin reactions, it was decided to attempt control of the diabetes without insulin. The patient was discharged on March 31, 1947, on a diet of protein 70 Gm.; fat 125 Gm.; and carbohydrate 200 Gm. She, however, did not regain her former strength and working capacity and by April 4, 1947, had lost weight from 89 pounds to 83 pounds. A 24-hour urine specimen on April 4, 1947 contained 70 Gm. of glucose. Readmission to the hospital was advised to institute insulin therapy.

On admission to the hospital May 13, 1947, the patient was continued on the diet of protein 70 Gm.; fat 125 Gm.; and carbohydrate 200 Gm.; and globin insulin, 4 units daily before breakfast, was started. This was gradually increased to 14 units daily. During the ten day hospital stay the

patient gained from 77 pounds to 89 pounds and no hypoglycemic reactions occurred. The 24-hour output of glucose in the urine fell to 20 Gm. The patient was discharged from the hospital on May 23, 1947 (Fig. 2).

In the past three months the patient has felt well, has done all her housework and gained weight to 96 pounds. The present insulin dosage is

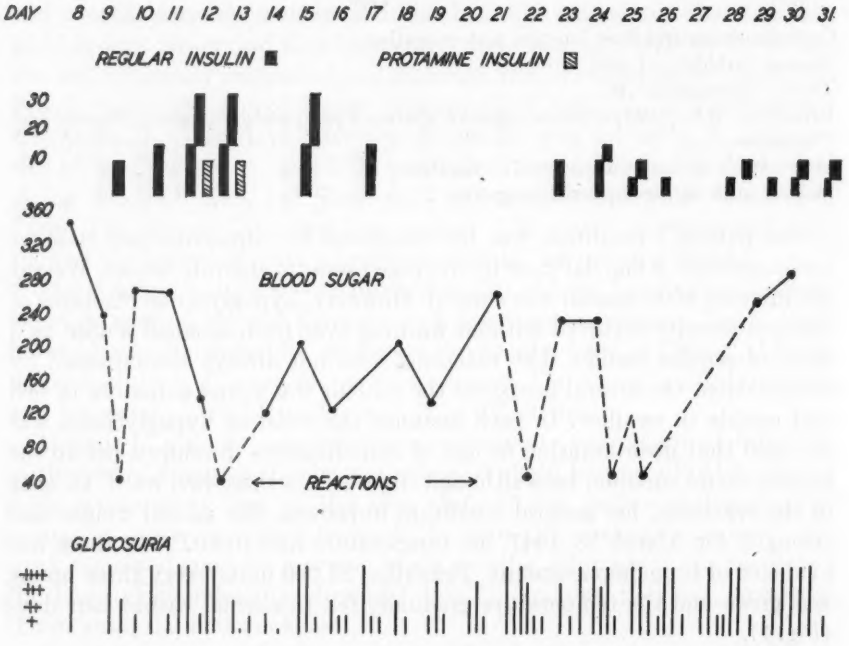

FIG. 1. Frequent severe hypoglycemic reactions during administration of regular insulin and of protamine zinc insulin in a patient with Addison's disease complicated by diabetes mellitus. (In this figure and in Figure 2, the ordinate numbers pertaining to insulin indicate the dose in units: those pertaining to blood sugar indicate mg. per 100 cc.)

12 units of globin insulin before breakfast. She has had one insulin reaction to this dosage. On July 12, 1947, the patient was brought to the hospital in hypoglycemic coma and was promptly revived by intravenous glucose. The preceding evening the patient had been moving furniture in preparation for redecorating her home.

On January 4, 1948 the urinary neutral 17-ketosteroids in twenty-four hours measured 0.5 mg. In the determination of the neutral ketosteroids the urine was hydrolyzed and fractionated according to Pincus (18); and

ketosteroids, separated by means of Girard's reagent (fraction 12), were determined by two-color spectrophotometry.[2]

COMMENT

In this patient, Addison's disease preceded the onset of diabetes mellitus by $2\frac{1}{2}$ years. Three similar cases have been reported in the literature.

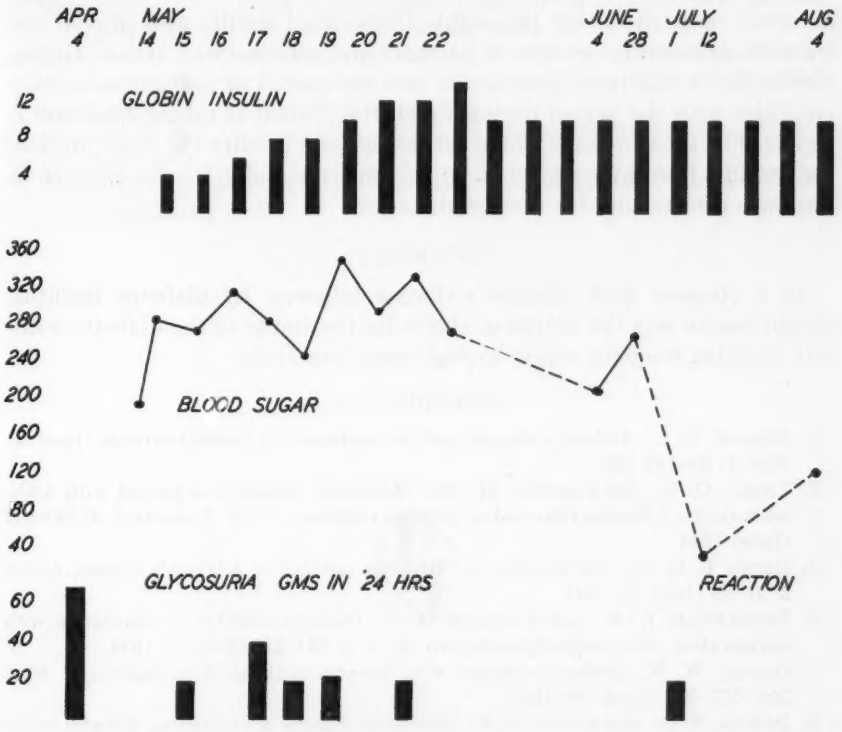

FIG. 2. Infrequent hypoglycemic reactions during administration of globin insulin in same patient shown in Figure 1.

Adrenal cortical hormone was administered only during the first seventeen days of therapy of the Addison's disease. Subsequently control was maintained with injections of desoxycorticosterone and later by pellets.

The onset of diabetes was abrupt and characterized by weakness, weight loss, polyuria and polyphagia. In spite of this and the high blood sugar and

[2] For this determination we are indebted to Mr. William T. Beher, Fellow in Biochemistry at the Research Institute of this hospital.

pronounced glycosuria, the patient did not have severe ketosis at any time. The blood CO_2 was only slightly decreased and diacetic acid was not found in the urine, although acetone was present.

The decision to use globin insulin resulted from the unsatisfactory and unpredictable results obtained from either unmodified or protamine zinc insulin. Protamine zinc insulin, with its maximum effect during the meal-free night hours, was obviously unsuitable and produced a severe hypoglycemic reaction during the night. Unmodified insulin also proved unsuitable, apparently because of its short and concentrated action. Globin insulin has a relatively slow action and the period of maximum activity coincides with the period during which the patient is taking food and is awake. For these reasons globin insulin seemed to offer the most promise for freedom from hypoglycemia. In this one case globin insulin apparently has been a more suitable therapeutic agent.

SUMMARY

In a Negress with Addison's disease followed by diabetes mellitus, globin insulin was the insulin of choice for treatment of the diabetes without resulting frequent severe hypoglycemic reactions.

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DIABETES IN A DWARF. A CASE REPORT

HERBERT SCHREIER, M.D. AND ISAAC LEWENTER, M.D.
From the Endocrine Clinic at Gouverneur Hospital, New York City

THE case presented is one of a juvenile diabetic who survived the preinsulin era. Her stunted growth gave rise to speculation on the "endocrine" stigmata of her dwarfism, whereas the starvation management of her diabetes in the preinsulin era was probably the main contributory factor. The course of her diabetes, complicated by hyperthyroidism, presents some points of interest.

CASE HISTORY

R.G., a 45-year-old white female, had no familial history of diabetes or dwarfism (Fig. 1). One older normally built sister suffered from rheumatoid arthritis; her rather

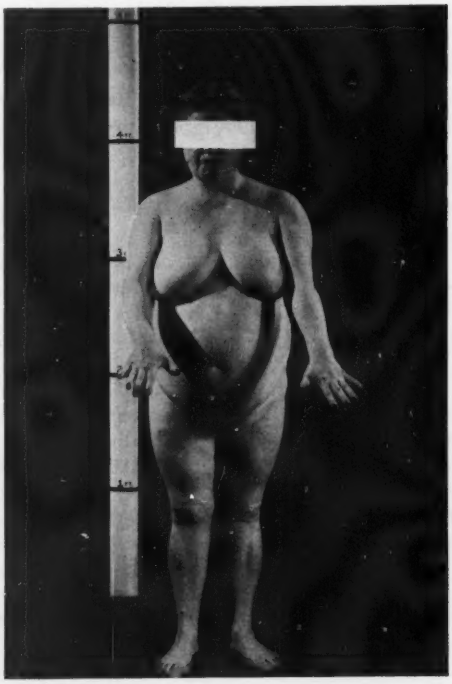

FIGURE 1

tall brother, from night blindness. The patient, the youngest of the three children, was born when her mother was 51 years old. She had an uneventful childhood, although

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she was sickly and could not follow a regular school curriculum. At the age of one year she had pneumonia. Menstruation started at eleven years, and was regular until November 1946, when the menopause ensued.

The diagnosis of diabetes was made at eleven years of age. Since this was in the pre-insulin era, the patient was subjected to prolonged hospital observation and starvation regimens at many hospitals. Insulin was administered to her for the first time in 1922, at the age of twenty, following diabetes of nine years duration.

The patient has been under observation in the diabetic clinic at Gouverneur Hospital since 1934, at which time the daily dose of insulin was 30 units.

In 1937, clinical evidence of hyperthyroidism developed, indicated by extreme nervousness, tachycardia, tremor of the hands and elevation of the basal metabolic rate to plus 32 per cent. The daily insulin dose had to be increased to 140 units. Thyroidectomy was recommended, and subtotal thyroidectomy was performed at Post-Graduate Hospital in January 1938. After an uneventful postoperative course, the patient was discharged from the hospital on a low caloric diet and on the same daily dose of insulin (140 units). On February 21, 1938, one month postoperatively, the B.M.R. was plus 15 per cent.

In April 1938 the patient started to gain weight, the hair on her head began to fall out, and she started complaining of paraesthesias. The B.M.R. fell, until on August 1, 1938 it was minus 1 per cent, and two weeks later minus 15 per cent. The insulin dose remained the same, 140 units daily.

Since 1938, the patient's attendance at the diabetic clinic at Gouverneur Hospital was more regular. The carbohydrate content of her diet through that period was 150 Gm. (Protein—60 Gm., Fats—60 Gm.). The insulin dosage administered up to date remains high: 70 units of protamine zinc insulin and 35 units of crystalline zinc insulin.

In January 1947 the patient started showing signs and symptoms of recurrent hyperthyroidism: loss of weight, exophthalmos, tremor, tachycardia (pulse rate 110/min.), a B.M.R. of plus 30 per cent, and two small nodules in the remaining thyroid. The RBC was 3,880,000; and the WBC, 8,505. Differential count: polymorphs—38 per cent, lymphocytes—49 per cent, monocytes—8 per cent, eosinophiles—4 per cent, basophiles—1 per cent. During treatment with propylthiouracil, pyridoxine, and feosol at the Post-Graduate Hospital Clinic, she responded very slowly; finally she showed a remission in June 1947. At present (Oct. 1947) she has a normal basal metabolic rate and is still on a maintenance dose of 25 mg. of propylthiouracil twice daily.

There has been no history of any insulin reaction or diabetic acidosis during the entire course of observation.

Physical Findings: Height—4 ft. 5 in.; weight—118½ lbs. Pronounced obesity with fat distributed predominantly in the breasts and trunk, and in the large abdominal apron. Hands and feet, particularly the fingers and toes, were short, small and thin. Hair distribution normal. Slight exophthalmos. Blood pressure, 120/80. Pulse rate, 80/min.

Essential Laboratory Findings: Blood sugar levels ranged between 200 and 300 mg. per 100 cc. The most recent report showed 200 mg. per 100 cc. The urine had never been found sugar free, although quantitative excretion of sugar in 24 hours had varied. The blood count was normal. Blood chemistry showed a non-protein nitrogen of 30 mg. per 100 cc.; free cholesterol, 159 mg. per 100 cc.; and cholesterol esters, 101 mg. per 100 cc.

X-ray study revealed a normal sella turcica with normal vascular markings. The suture lines appeared to have closed prematurely. Some prominence of convolutional markings was observed. The pineal body was not visualized. Long bones were normal.

DISCUSSION

Type of Dwarfism: In the differential diagnosis of the type of dwarfism in our case, we may, a priori, rule out some types of secondary dwarfism with characteristic features completely different from the signs shown by the patient. In this category belong osteopsathyrosis, achondroplastic dwarfism, and dwarfism due to renal rickets, congenital disorders or chronic infections.

The three types of dwarfism to be considered are: (a) primary, (b) pituitary and (c) diabetic dwarfism. Primary dwarfism involves a defect in the constitutional growth process on a hereditary basis (1, 2). This element cannot be found in our patient, who shows externally all the signs of a pituitary dwarf (3, 4), with one very important and decisive exception. She is sexually well developed; whereas the characteristic feature of pituitary dwarfism is infantilism of the primary sex organs and amenorrhea.

In physical appearance, diabetic dwarfs resemble pituitary dwarfs. The sella turcica, however, is normal, and sexual hypoplasia and amenorrhea are not present. Dwarfism in diabetics develops in childhood, not as a result of the diabetes as such, or because of other endocrine disturbances, but rather because of faulty control of the diabetes and malnutrition (5, 6, 7). Our patient developed diabetes long before insulin was discovered, and in the interim the disease was not well controlled. The presence of uncontrolled diabetes, accompanied by poor nutrition, probably caused retardation of growth. It would seem logical, therefore, to accept the diagnosis of diabetic dwarfism, due to uncontrolled diabetes in childhood (8).

Some Special Features of Diabetes in the Described Patient: Despite high doses of insulin there were no insulin reactions. Although she was a juvenile diabetic, the patient did not show signs of diabetic ketosis. The mildness of the diabetes in this young girl, if attributed to hypopituitarism, would imply Houssay's phenomenon in a human, but insulin resistance does not confirm this impression.

It is interesting that thyroidectomy changed neither the insulin requirement nor the clinical features of the diabetes.

SUMMARY

1. A 45-year-old diabetic dwarf, who had lived for nine years in the preinsulin era, has been presented. Hyperthyroidism developed as a complication in her thirty-fifth year.

2. In this type of dwarfism, endocrine factors other than the pancreatic diabetes were formerly over-stressed.

3. Response to insulin was unlike that of a juvenile diabetic in that there was no ketosis and no insulin reactions.

4. Despite the successful treatment of the hyperthyroidism, large doses of insulin were continuously required.

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PROGRAM OF THE THIRTIETH ANNUAL MEETING OF THE ASSOCIATION FOR THE STUDY OF INTERNAL SECRETIONS

The Thirtieth Annual Meeting of the Association for the Study of Internal Secretions will be held in the Palmer House, Chicago, Illinois, June 18 and 19, 1948.

The scientific sessions will be held in the Red Lacquer Room and registration will be on the fourth floor just outside the Red Lacquer Room. The Annual Dinner will be held in the same room on Friday, June 18th at 7 P. M. and will be preceded by a cocktail party, the location of which will be announced later.

All members of the Association who plan to attend the Thirtieth Meeting are urged to make their reservations at once with the Palmer House, stating the time of arrival and how long they plan to remain in Chicago.

PROGRAM

FRIDAY, JUNE 18, 1948

8:30 A.M. Registration

I. 9:30 A.M. Red Lacquer Room

J. S. L. BROWNE, *presiding*

1. PSEUDO-GLANDULAR DISTURBANCES.
   by Hugo R. Rony
2. SYNDROME OF CYPTORCHIDISM, HEART DISEASE AND DERMATOSIS.
   by S. J. Glass
3. CONSTITUTIONAL PRECOCIOUS PUBERTY CONTROLLED BY ANDROGEN THERAPY.
   by S. Charles Freed and Minnie Goldberg
4. A STUDY OF THE BIOLOGICAL ACTIVITY AND THE MAGNITUDE OF ENDOGENOUS ANDROGEN PRODUCTION IN A CASE OF ANDRENOGENITAL SYNDROME.
   by Anne C. Carter and Ephraim Shorr
5. PSEUDOHERMAPHRODISM. EARLY AND LATE RECOGNITION.
   by M. James Whitelaw
6. CLINICAL, LABORATORY, OPERATIVE AND POSTMORTEM OBSERVATIONS IN IN-

FANTS AND CHILDREN WITH MULTIPLE CONGENITAL MALFORMATIONS (TURNER'S SYNDROME, OVARIAN AGENESIS AND RELATED COMBINATIONS).
by Frank L. Plachte (introduced by Henry H. Turner)

7. A SYNDROME CHARACTERIZED BY HYPERCALCEMIA. CALCINOSIS, AND RENAL INSUFFICIENCY FOLLOWING PROLONGED INTAKE OF CALCIUM AND ALKALI.
by Charles H. Burnett, Robert R. Commons (by invitation), Fuller Albright and John E. Howard
8. HYPOPARATHYROIDISM, WITH MENTAL TROUBLES AND ECTODERMAL DISORDERS.
by Manuel Villaverde
9. TREATMENT OF FAR ADVANCED INOPERABLE CARCINOMA OF THE BREAST WITH ESTROGENS AND ANDROGENS.
by Samuel G. Taylor, III, Danely Slaughter (by invitation) and Frederick W. Preston (by invitation)
10. HORMONAL FACTORS INVOLVED IN THE REGULATION OF BODY TEMPERATURE DURING MENSTRUAL CYCLE AND PREGNANCY.
by Charles L. Buxton and William B. Atkinson
11. THE EFFECTS OF CERTAIN STEROIDS—INTRAMUSCULAR AND SUBLINGUAL—ON THE BASAL BODY TEMPERATURE OF THE ADULT HUMAN MALE.
by Robert M. Perlman

II. 2:00 P.M. Red Lacquer Room

C. N. H. LONG, *presiding*

12. A SIMPLIFIED HYPOPHYSECTOMIZED RAT ADRENAL ASCORBIC ACID BIOASSAY METHOD FOR ADRENOCORTICOTROPHIN (A.C.T.H.); SPECIFICITY AND APPLICATION TO PREPARATIVE PROBLEMS.
by Paul L. Munson, Alfred G. Barry, Jr. (by invitation), and F. C. Koch
13. CONTENT OF ADRENOCORTICOTROPHIC HORMONE (A.C.T.H.) IN THE RAT PITUITARY UNDER OPTIMAL AND STRESSFUL ENVIRONMENTAL CONDITIONS.
by George Sayers, Marshal Merkin (by invitation) and J. N. Tortoreto (by invitation).
14. THE ACTIVATION OF THE ADRENAL CORTEX BY INSULIN HYPOGLYCEMIA.
by H. Gershberg (by invitation) and C. N. H. Long.
15. INFLUENCE OF ADRENOTROPHIC HORMONE ON SODIUM EXCRETION IN HYPOPHYSECTOMIZED RATS.
by Betty L. Rubin (by invitation) and Ralph I. Dorfman
16. FACTORS INFLUENCING THE CORTICOTROPHIN PRODUCTION OF THE ANTERIOR PITUITARY.
by Hans Selye
17. THE USE OF ANDRENOCORTICOTROPHIN AS A TEST OF ADRENAL CORTICAL RESERVE.
by George W. Thorn, Peter H. Forsham (by invitation), Lillian Recant (by invitation) and A. Gorman Hills (by invitation)
18. OBSERVATIONS ON THE PITUITARY-ADRENAL RESPONSE FOLLOWING EPINEPHRINE INFUSION IN MAN.
by Lillian Recant (by invitation), Peter H. Forsham (by invitation) and George W. Thorn
19. FATE AND METABOLIC ACTION OF INTRAVENOUSLY ADMINISTERED ADRENOCORTICOTROPHIC HORMONE (A.C.T.H.).
by Thomas W. Burns (by invitation), George Sayers, Frank H. Tyler (by

invitation), B. V. Jager (by invitation), T. B. Schwartz (by invitation), Emil L. Smith (by invitation) and L. T. Samuels

20. Metabolic Changes Following the Administration of Pituitary Adrenocorticotrophic Hormone (A.C.T.H.) to Normal Humans.
by H. T. McAlpine (by invitation), E. H. Venning, L. Johnson (by invitation), V. Schenker (by invitation), M. M. Hoffman and J. S. L. Browne
21. The Effect of Adrenocorticotrophin on Antibody Levels in Normal Human Subjects.
by P. H. Herbert and J. A. de Bries (introduced by J. S. L. Browne)
22. A Comparison of the Effect on Bone Formation of the Hyperadrenocorticism of Cushing's Syndrome with that Induced by Adrenocorticotrophic Hormone (A.C.T.H.).
by Frederic C. Bartter (by invitation), Anne P. Forbes and Fuller Albright
23. Adrenal Cortical Unresponsiveness in Patients with Gastric Cancer.
by Edward C. Reifenstein, Jr., N. F. Young (by invitation), Aurelia Potor (by invitation), Benedict Duffy (by invitation) and F. Homburger (by invitation)
24. The Excretion of Adrenal Metabolites in Human Urine.
by Konrad Dobriner, Seymour Lieberman (by invitation) and C. P. Rhoads (by invitation)

III. Annual Dinner, Friday, June 18.

7:30 p.m.—Red Lacquer Room, Palmer House

Presentation of E. R. Squibb and Sons Award for 1948
Presentation of Ciba Award for 1948
Presentation of Ayerst, McKenna and Harrison Fellowship for 1948.
by Warren O. Nelson, Chairman of the Committee on Awards 1947–48

President's Address: C. N. H. Long, Yale University

Saturday, June 19, 1948

IV. 9:00 a.m. Red Lacquer Room

R. G. Hoskins, *presiding*

25. Preparation of Crystalline Growth Hormone.
by Jacob B. Fishman (by invitation), Alfred E. Wilhelmi (by invitation) and Jane A. Russell
26. The Influence of Purified Growth Hormone on Fasting Metabolism.
by Clara M. Szego and Abraham White
27. Unpredictable Effects of Growth Hormone Preparations on Nitrogen Storage.
by Paul Bartlett (by invitation) and Oliver H. Gaebler
28. Studies in Growth. I. The Effects of Androgen in Gigantism and Acromegaly.
by Laurence W. Kinsell, George D. Michaels (by invitation), Choh Hao Li (by invitation) and William E. Larsen (by invitation)
29. The Effect of Iodine Injections on Energy Metabolism and Plasma Protein-Bound Iodine of Rats.
by S. B. Barker and H. J. Lipner (by invitation)

30. The Effect of Pituitary and Non-Pituitary Gland Factors on the Formation of Intracellular Colloid Droplets in the Thyroid Gland in Vivo and in Vitro.
by Samuel Dvoskin
31. Inactivation of the Exophthalmic, Thyrotropic and Ketogenic Principles of Anterior Pituitary Extract by Iodination.
by William McK. Jefferies
32. Newer Methods of Antagonizing Hyperthyroidism.
by Robert H. Williams, Rene F. Tagnon (by invitation), Herbert Jaffe, (by invitation), Beverly T. Towery (by invitation) and Walter F. Rogers (by invitation)
33. The Use of Radioactive Iodine (I 131) in the Study of Normal and Disordered Thyroid Function in Man.
by Sidney C. Werner and Edith Quimby (by invitation)
34. The Effect of Thyroid Stimulating Hormone on the Function of Human Normal and Malignant Thyroid Tissue.
by J. B. Trunnell (by invitation), R. W. Rawson, L. D. Marinelli (by invitation) and Ruth Hill (by invitation)
35. The Relation Between Infant Birthweight and Subsequent Development of Maternal Diabetes Mellitus.
by Joseph P. Kriss and Palmer H. Futcher (introduced by Cyril M. MacBryde)

V. 2:00 p.m. Red Lacquer Room

A. T. Kenyon, *presiding*

36. Absorption and Excretion of Chorionic Gonadotrophin When Administered Intramuscularly to Women.
by J. T. Bradbury and Willis E. Brown
37. The Renal Clearance of Chorionic Gonadotrophic Hormone in Pregnancy and in Neoplasm of the Testis.
by C. F. Gastineau (by invitation), A. Albert and L. M. Randall (by invitation)
38. The Metabolic Response to Chorionic Gonadotrophin in Young Men.
by Kathryn Knowlton (by invitation) and Allan T. Kenyon
39. Blood Gonadotrophin Studies During Pregnancy in Relation to the Fetal Sex.
by H. E. Nieburgs and Robert B. Greenblatt
40. On the Principal Estrogenic Constituents of the Urine of the Stallion.
by Louis Levin
41. Mechanism of Inactivation of α-Estradiol by Rat Liver in Vitro.
by R. H. deMeio (by invitation), A. E. Rakoff, A. Cantarow and K. E. Paschkis
42. Cozymase in the Hepatic Inactivation of α-Estradiol.
by Richard L. Coppedge (by invitation), Albert Segaloff, Herbert Sarett (by invitation) and Aaron Altshul (by invitation)
43. Interference with Estrogen-Induced Growth in the Female Genital Tract by Folic Acid.
by Roy Hertz
44. The Relation of Folic Acid to the Action of Estrogens.
by Irene T. Kline (by invitation) and Ralph I. Dorfman

45. Fluorescent Phenomena of the Vulva Associated with Sex Hormone Metabolism.
by M. Sydney Margolese
46. Testicular Deficiency: A Clinical and Pathological Study.
by R. Palmer Howard, Ronald C. Sniffen (by invitation) and Fred A. Simmons
47. A Comparison of the Effect of Various Androgens on the Temporal Muscle and Organs of the Castrated Male Guinea Pig.
by Charles D. Kochakian and Jane Harrison Humm (by invitation)

VI. Annual Business Meeting

5:00 p.m. Red Lacquer Room

Papers to be Read by Title

48. The Use of Whole Adrenal Cortical Extract in Experimental Infections.
by Erwin P. Vollmer, James D. Gillmore (by invitation), Leo Cravitz (by invitation) and J. E. Samsell (by invitation).
49. The Work Performance of Adrenalectomized Rats Given Continuous Intravenous Infusions of Glucose.
by Dwight J. Ingle and James E. Nezamis (by invitation)
50. Sublingual Administration of Desoxycorticosterone Acetate in the Treament of Addison's Disease.
by Evelyn Anderson, Lawrence W. Kinsell, Troy C. Daniels (by invitation) and Edward Henderson
51. Excretion of Adrenal Metabolites Following the Administration of Adrenocorticotrophic Hormone to Normal Human Subjects.
by Eleanor H. Venning, V. E. Kazmin (by invitation), Miriam Ripstein (by invitation), H. T. McAlpine (by invitation) and M. M. Hoffman
52. The Effect of 11-Dehydrocorticosterone on Fecal Fat Excretion.
by Grace E. Bergner (by invitation), Roger A. Lewis (by invitation), Frances W. Stout (by invitation), George W. Thorn and Kendall Emerson, Jr.
53. An Estimation of the Quantity of 11-17-Oxysteroid Excretion by the Human Adrenal Stimulated by ACTH.
by A. Gorman Hills (by invitation) and George W. Thorn
54. Isolation of Urinary Steroids from a Patient with Apparent Adrenal Involvement.
by A. M. Miller (by invitation) and Ralph I. Dorfman
55. The Effect of Adrenalectomy and Desoxycorticosterone Acetate Administration upon the ECG Response of the Rat to Cardiac Glycosides.
by Herbert S. Kupperman, Joseph G. Benton (by invitation) and Arthur C. DeGraff (by invitation)
56. Activation of the Adrenal Cortex in Human Subjects Following Electroconvulsive Therapy (E.C.T.) and Psychomotor Stress.
by R. A. Cleghorn and A. J. Goodman (by invitation), B. F. Graham, M. H. Jones and N. K. Rublee
57. Effect of Adrenal Cortical Compounds on Electrolyte Metabolism

OF A PATIENT WITH ADDISON'S DISEASE DURING HIGH SODIUM CHLORIDE INTAKE.
by Aurelia Potor (by invitation), Nelson F. Young (by invitation), F. Homburger (by invitation) and Edward C. Reifenstein, Jr.

58. NITROGENSPARING (PROTEIN-ANABOLIC) ACTION OF THYROID HORMONE.
by J. Rupp (by invitation) and K. E. Paschkis

59. THIOURACIL EFFECT ON PLASMA AND LIVER PROTEIN CONCENTRATIONS.
by James H. Leathem

60. RADIOIODINE UPTAKE BY THE THYROID AS AN AID IN DIFFERENTIAL DIAGNOSIS.
by S. M. Seidlin, E. Oshry (by invitation), I. Rossman (by invitation) and L. Leiter (by invitation)

61. THYROID UPTAKE OF RADIOACTIVE IODINE IN THE NORMAL AND HYPOMETABOLIC HUMAN.
by Martin Perlmutter (by invitation) and Peter H. Forsham (by invitation)

62. CELLULAR INVOLUTION IN THE THYROID.
by Nathan B. Friedman

63. METHYL THIOURACIL IN THE TREATMENT OF THYROTOXICOSIS.
by Grosvenor W. Bissell, John M. Benny (by invitation), Victor Totah (by invitation) and Florence Gilbert (by invitation)

64. MODIFICATION OF THE ESTRUAL CYCLE OF THE EWE BY THE USE OF PROGESTERONE; THE EFFECT UPON SUBSEQUENT OVULATION RATE AND FERTILITY OF OVA.
by R. H. Dutt (by invitation) and L. E. Casida

65. EFFECTS OF VARIOUS ESTROGENIC PREPARATIONS ON THE VAGINAL MUCOSA.
by Mildred Vogel (by invitation), Thomas H. McGavack and Joseph Mellow (by invitation)

66. THE USE OF THE VAGINAL SMEAR IN THE ASSAY OF ESTROGENS GIVEN ORALLY OR INTRAMUSCULARLY.
by Willis E. Brown and J. T. Bradbury

67. THE SIMILARITY OF ESTROGENIC EFFECT IN PREMENSTRUAL TENSION, MENSTRUAL ANOMALIES, CHRONIC CYSTIC MASTITIS AND CANCER OF THE BREAST.
by Joseph H. Morton

68. HYPERESTROGENISM TREATED WITH LACTOGENIC HORMONE (PROLACTIN).
by Manuel Villaverde

69. THE FACTOR OF RHYTHM IN EXPERIMENTAL MENSTRUATION.
by Doris H. Phelps

70. HORMONAL PELLETS IN THE MANAGEMENT OF THE MENOPAUSAL SYNDROME.
by Robert B. Greenblatt and Roland R. Suran (by invitation)

71. THE EFFECT OF HYPOPHYSECTOMY ON THE OVULABILITY OF THE OVARIAN FOLLICLE OF THE DOMESTIC HEN.
by Irving Rothchild and R. M. Fraps

72. PROGNOSTIC VALUE OF PREGNANEDIOL EXCRETION IN THREATENED ABORTION WITH SPECIAL REFERENCE TO THE EFFECTS OF DIETHYLSTILBESTROL.
by A. R. Abarbanel

73. FURTHER STUDIES ON THE ENDOMETRIAL CUPS OF THE PREGNANT MARE.
by H. H. Cole and G. H. Hart

74. INACTIVATION OF POSTERIOR PITUITARY ANTIDIURETIC HORMONE OF THE LIVER.
by W. J. Eversole, J. H. Birnie (by invitation) and Robert Gaunt

75. THE ESTIMATION OF DEHYDROISOANDROSTERONE AND RELATED COMPOUNDS

IN HUMAN URINE BY A MODIFICATION OF THE PETTENKOFFER REACTION.
by Richard L. Landau and Kathleen Lugibihl (by invitation)

76. CLINICAL EVALUATION FOR 17-KETOSTEROIDS BY THE RAPID METHOD.
by T. H. McGavack, S. Kenigsberg, A. M. Shearman and K. J. Drekter

77. THE APPLICATION OF PAPER PARTITION CHROMATOGRAPHY TO KETOSTEROIDS.
by Robert B. Burton (by invitation), Alejandro Zaffaroni (by invitation) and E. Henry Keutmann

78. A RAPID MODIFICATION OF THE ZIMMERMANN TEST FOR KETOSTEROIDS.
by Sidney Pearson and Sylvester Giaccone (by invitation)

79. A FLUORIMETRIC METHOD FOR THE DETERMINATION OF ESTRONE AND ESTRADIOL IN HUMAN URINES.
by Joseph W. Jailer

80. A. CLINICAL BIO-ASSAY FOR CHORIONIC GONADOTROPHIN.
by A. Albert

81. SPECIFICITY OF A COLORIMETRIC METHOD FOR DEHYDROISOANDROSTERONE IN URINE EXTRACTS.
by Paul L. Munson, Mary Ellen Jones (by invitation), Philip J. McCall (by invitation) and T. F. Gallagher (by invitation)

82. THE COLORIMETRIC DETERMINATION OF SODIUM AND ITS APPLICATION IN THE STUDY OF SODIUM AND CHLORIDE BALANCE.
by Joseph W. Goldzieher and Gilbert Stone (by invitation)

83. DEPRESSION OF LYMPHOCYTE COUNT AFTER ORALLY ADMINISTERED GLUCOSE.
by Paul A. Marks (by invitation), Dorothy T. Marks (by invitation) and Joseph W. Jailer

84. PITFALLS IN THE DIAGNOSIS OF DIABETES.
by Bernard A. Watson

85. ELECTROLYTE BALANCE STUDIES IN THE UNCONTROLLED AND CONTROLLED DIABETIC STATE.
by Jonas Weissberg (by invitation), Thomas H. McGavack, A. M. Shearman (by invitation) and I. J. Drekter

86. THE ROLE OF THE ENDOCRINE GLANDS IN BODY TEMPERATURE REGULATION.
by H. E. Nieburgs and Robert B. Greenblatt

87. THE LIPOGENIC ACTIVITY OF PROPYLTHIOURACIL, TRIPHENYLCHLOROETHYLENE AND HEXESTROL IN CHICKENS.
by R. George Jaap

88. THE INFLUENCE OF THE PITUITARY GLAND ON BLOOD VESSEL DEVELOPMENT. THE EFFECT OF CONCENTRATED PITUITARY EXTRACT ON THE ISOLATED KIDNEY.
by Robert C. Moehlig and Louis Jaffe (by invitation)

89. RESULTS OF PROLONGED MEDICAL TREATMENT OF OBESITY WITH DIET ALONE, DIET AND THYROID PREPARATIONS, AND DIET AND AMPHETAMINE.
by David Adlersberg and Martin E. Mayer (by invitation)

90. THE URETHRAL SMEAR IN THE NORMAL HUMAN MALE.
by Mildred T. Vogel (by invitation), Thomas H. McGavack and Henry Kammandel (by invitation)

91. THE SPECIFIC OVARIAN HYPEREMIC INDUCING EFFECT OF LUTEINIZING AND LUTEOTROPHIC HORMONES.
by Herbert S. Kupperman, W. H. McShan and Roland K. Meyer

ANNOUNCEMENT OF THE 1948 LAURENTIAN HORMONE CONFERENCE

The Laurentian Hormone Conference of the A.A.A.S. will meet in 1948 at the Forest Hills Hotel, Franconia, New Hampshire, from September 13th to 18th inclusive. The program (which will be published in full at a later date) will consist of four sections:

I. The metabolism of steroid hormones *in vivo* and *in vitro*.
II. Thyroid physiology and function.
III. The role of hormones in tissue and body metabolism.
IV. Hypothalamic neuro-humoral relationships.

Because of limited accommodations, attendance is by invitation, but the Committee on Arrangements will receive applications for membership until June 15, 1948. Applications should be addressed to: Dr. Gregory Pincus, Chairman, Committee on Arrangements, 222 Maple Avenue, Shrewsbury, Massachusetts.